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(54) Title: METHODS FOR REGULATION OF INSULIN-LIKE GROWTH FACTOR I RECEPTOR (57) Abstract The present invention provides nucleotide sequences and methods for increasing expression of type I insulin-like growth factor receptor, in particular to promote healing of burns, broken bones, and other wounds, nerve regeneration in injured tissue and angiogenesis. Inhibition of angiogenesis and/or vascular smooth muscle cell growth and proliferation can be achieved using IGF IR-specific antisense RNAs or oligonucleotides. IGF IR ATG-directed sense oligonucleotides elevate the expression of the IGF IR gene.		

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METHODS FOR REGULATION OF INSULIN-LIKE
GROWTH FACTOR I RECEPTOR

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Field of the Invention

The present invention relates to the use of oligonucleotide-mediated methods for the regulation of growth factor receptor gene expression, particularly insulin-like growth factor 1 receptor, and methods for wound healing, promoting blood vessel proliferation, preventing postangioplasty restenosis and/or post transplant atherosclerosis, and regulating smooth muscle cell growth.

Background of the Invention

Insulin-like growth factor I (IGF I) is a ubiquitous peptide which regulates growth and differentiation in many cell types. IGF 1, which is synthesized and secreted by vascular smooth muscle cells, acts as an autocrine/paracrine factor. IGF 1 acts to stimulate progression of cells from the G1 phase to the S phase of the cell cycle. Thus, IGF 1 serves a critical role in cell growth and proliferation and in normal development.

The effects of IGF are mediated by the type 1 IGF receptor (IGF IR), which is a membrane tyrosine kinase (Ullrich et al. (1986) *EMBO J.* 5:2503-2512; Werner et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:7451-7455). The IGR IR is a glycosylated heterotetramer which consists of two extracellular α subunits and

two transmembrane β subunits which each have extracellular and intracellular portions. The α subunits contain the growth factor binding site. Several growth factors including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and Angiotensin II (Ang II) upregulate vascular smooth muscle cell (VSMC) IGF IR (Pfeile et al. (1987) *Endocrinology*, 120:2251-2258; Ververis et al. (1993) *Circulation Research*, 72:1285-1292). Pre-incubation of VSMC with bFGF leads to increased mitogenic responsiveness to IGF I (Ververis et al. (1993) *supra*). It has been shown that antisense targeting of the IGF IR inhibits growth of SV-40 transformed BALB/c3T3 fibroblasts (Porcu et al. (1992) *Mol. Cell. Biol.* 12:5069-5077). Furthermore, overexpression of the IGF IR has been shown to induce transformation of NIH/3T3 cells (Kaleko et al. (1990) *Mol. Cell. Biol.* 10:464-470).

Anti-IGF antiserum inhibits PDGF-mediated growth of VSMC (Clemmons et al. (1988) *J. Clin. Invest.* 75:1914-1918). Angiotensin II (Ang II), a vasoactive and mitogenic peptide, transcriptionally regulates the IGF I gene in VSMC, and neutralization of extracellular IGF I with an anti-IGF I antibody inhibits ang II-induced DNA synthesis in VSMC (Delafontaine et al. (1993) *J. Biol. Chem.* 268:16866-16890).

Summary of the Invention

The present invention provides methods for regulating cell growth, in particular vascular smooth muscle cell growth, and methods for stimulating proliferative process including, but not limited to, wound healing, burn healing, bone healing, nerve regeneration, and angiogenesis. Negative regulation of vascular smooth muscle cell growth and proliferation allows for the prevention or treatment of medical conditions including, but not limited to, coronary artery disease, post-angioplast restenosis, post coronary transplant atherosclerosis, atherosclerotic lesions and vascular complications of hypertension, including hypertensive retinopathy, hypertensive nephropathy, cerebrovascular disease and myocardial hypertrophy, and in the treatment of solid cancers.

Negative regulation of cell proliferation in particular of vascular smooth muscle cells is achieved using antisense oligonucleotides, which oligonucleotides are at least about 14 nucleotides, and preferably about 18 to about 22 nucleotides, in length and comprises contiguous sequence complementary to at least a portion of the mRNA encoding a growth factor receptor, for example, type I insulin-like growth factor receptor (IGF IR). Preferably, the antisense oligonucleotide, is complementary to the growth factor receptor mRNA sequence in the vicinity of the translation start site of the coding sequences. However, other antisense sequences complementary to coding sequence information is effective for inhibiting factor receptor gene expression, thereby inhibiting target cell growth and proliferation. Antisense nucleic acid molecules can also be made as transcription products from non-naturally occurring recombinant nucleic acid molecules expressed in the target tissue. Preferably, expressed antisense RNAs are at least about 100 nucleotides in length and up to 1 Kb or more. Specifically exemplified IGF IR antisense oligonucleotides useful in the present methods include those having sequences as given in SEQ ID NO:1 (ATG-directed) and SEQ ID NO: 4. Both these sequences and other antisense molecules complementary to IGF IR mRNA effectively inhibit IGF IR expression in rat and human cells, such as vascular smooth muscle cells, thereby preventing or ameliorating post angioplasty restenosis and post-transplant atherosclerosis of the coronary arteries, and inhibiting angiogenesis in solid tumors.

Another aspect of the present invention is the positive regulation of growth factor receptor gene expression via ATG-directed sense oligonucleotides and, as specifically embodied herein, for up-regulation of type I insulin-like growth factor receptor gene expression, with the result that cell growth and proliferation is stimulated. Thus, improved wound healing, burn healing, healing of broken bones, angiogenesis, nerve regeneration and the like is achieved when ATG-directed oligonucleotides are applied to or expressed in target tissue. Within this context, the target tissue can be wounded or burned.

tissue, a broken bone, a damaged nerve, an area of peripheral vascular disease, or cardiac tissue damaged by myocardial infarction. In the latter two examples, angiogenesis (development of collateral blood vessels) will ameliorate the damage. Within the scope of this aspect of the invention are ATG-directed sense oligonucleotides effective for stimulating growth factor receptor gene expression and, as specifically exemplified, an oligonucleotide comprising a sequence as given in SEQ ID NO: 2 is effective in human and rat cells and tissue. Functionally equivalent ATG-directed sense oligonucleotides can be readily produced, without the expense of undue experimentation, for other species using the teachings of the present disclosure taken with knowledge and techniques readily accessible to the art.

In the present methods based on inhibition of IGF IR expression, the antisense oligonucleotides can be chemically synthesized from ribonucleotide or deoxynucleotide precursors or they may be transcription products expressed from non-naturally occurring recombinant DNA molecules such as adenovirus or Epstein-Barr virus vectors. Preferably, such vectors do not persist in the tissues for very extended periods of time.

Preferred chemically synthesized sense or antisense oligonucleotides are those with chemical modifications which prolong persistence of intact molecules inside and outside cells, including, but not limited to, phosphorothioate, phosphorodithioate, phosphotriester, methylphosphonate, phosphoramidite, α -anomer and phosphoroselenoate oligonucleotides, the syntheses of which are known to the art.

Brief Description of the Figures

Figure 1 illustrates the effects of IGF IR ATG-directed oligonucleotides on basal and serum-induced DNA synthesis. VSMC were incubated in serum-free medium alone (SFM) or in the presence of antisense (AS), mismatch (M), or sense (S) oligonucleotides (SEQ ID Nos: 1, 3 and 2, respectively) (0.1-10 μ M) for 48 hr. [3 H]-Thymidine incorporation was then determined as basal levels (in the continued presence of SFM) and in response

to 10% calf serum (10% CS) as described in Example 3. Results are the mean \pm standard error (SE) of duplicate determinations from three to ten independent experiments for each condition.

Figure 2 illustrates the effects of IGF IR ATG-directed oligonucleotides on the proliferation of VSMC. 50% confluent VSMC were exposed to SFM, DMEM with 10% CS alone, or DMEM with 10% CS and increasing concentrations of AS, M or S oligonucleotides (SEQ ID NOS:1, 3 and 2, respectively) (0.1 - 10 μ M). Cell counts were determined at 96 hr. Shown is the mean \pm SE of duplicate measurements from four separate experiments.

Figure 3 illustrates the effects of IGF IR oligonucleotides on the mitogenic response to IGF I. VSMC were incubated for 48 hr in DMEM containing 10% CS alone or in the presence of 5 μ M AS or S oligonucleotides (SEQ ID NOS: 1 and 2, respectively). Cells were then exposed to fresh SFM containing increasing concentrations of human recombinant IGF I (0 - 50 ng/ml) for 24 hr and [3 H]-thymidine incorporation determined as described in Example 3. Results are the mean \pm SE of duplicate determinations from four separate experiments.

Figure 4 illustrates the effects of IGF IR oligonucleotides on mitogenic response to ang II. VSMC were incubated for 48 hr in DMEM containing 10% CS alone or in the presence of 5 μ M AS, M or S oligonucleotides (SEQ ID NOS: 1, 3 and 2, respectively). Cells were then exposed to fresh SFM containing increasing concentrations of ang II (0 - 1000 nM) for 36 hr. [3 H]-thymidine incorporation was determined as described in Example 3. Results are the mean \pm SE of duplicate measurements from three to seven experiments for each condition.

Figures 5A-5C illustrate the effects of ATG-directed IGF IR oligonucleotides on IGF IR and Angiotensin II (Ang II) receptor numbers. Vascular smooth muscle cells (VSMC) were incubated in SFM (Fig. 5A) or in DMEM with 10% CS (Figs. 5B-5C) alone (control), or in the presence of antisense (AS), missense (M) or sense (S) oligonucleotides (SEQ ID Nos. 1, 3 or 2, respectively) (5 μ M) for 48 hours 125 I-IGF I (Figs 5A, 5B) and [125 I-Sar¹-Ile⁶]-Ang II (Fig. 5C) displacement binding experiments were performed and data analyzed using the LIGAND program. Results shown are the

mean \pm (SE) of duplicate determinations from two to six separate experiments for each condition.

Figures 6A-6B illustrate the effects of non-ATG-directed sense (S) and antisense (AS) oligonucleotides (SEQ ID Nos. 5 and 4, respectively) on VSMC DNA synthesis and IGF IR number. In Fig. 6A, VSMC were incubated in SFM alone or in the presence of AS or S oligonucleotides (0.1 - 10 μ M) for 48 hours. [3 H]-thymidine incorporation was then determined basally (in the continued presence of SFM) or in response to 10% CS as described in Example 6. Results are the mean \pm standard error of duplicate measurements from three to eight experiments for each condition. In Fig. 6B, VSMC were incubated in 10% CS alone (control), or in the presence of 5 μ M AS or S oligonucleotide for 48 hours, and radiolabeled IGF I binding experiments were performed as described in Example 8. Results are the mean \pm SE of duplicate measurements from two separate experiments for each condition.

Figure 7 illustrates the effects of ATG-directed IGF IR antisense (AS), sense (S) and mismatch (M) oligonucleotides on IGF IR mRNA levels. VSMC were incubated for 48 hours in 10% CS alone (control) or in the presence of 5 μ M AS, M or S ODNs. Total RNA was extracted and co-hybridized using [32 P]-UTP labeled IGF IR and GAPDH antisense riboprobes. IGF IR mRNA levels (corrected for GAPDH) are shown as percent control (mean \pm SE, n=5).

Figure 8 diagrammatically illustrates the construction of episomal vector pAnti-IGF IR. The rat IGF IR cDNA clone p118 was digested by XhoI and KpnI and the 0.8 kb restriction fragment was ligated in an antisense orientation into pCEP4. CMV, cytomegalovirus immediate early gene enhancer/promoter; SVpA, simian virus 40 poly A; EBNA-1, EBV-encoded nuclear antigen 1; oriP, EBV origin of replication; Hyg, hygromycin resistance gene; Amp, ampicillin resistance gene; Km, kanamycin resistance gene.

Figure 9A-9B illustrate the effect of antisense IGF IR cDNA transcription on IGF IR number and on DNA synthesis. Figure 9A shows IGF IR number per 10^5 cells in CA9 antisense-expressing and control cells. Confluent and up to two days postconfluent monolayers of clone CA9 (transfected with pAnti-IGF IR, CA) and of clones ME8 and ME10 (transfected with vector alone, ME)

underwent ^{125}I -IGF I radioligand binding studies as described in Example 6 herein. Binding data were analyzed using the LIGAND program. Shown is the mean \pm SE of results from 8 separate experiments confluent and postconfluent monolayers of clone CA9 and clones ME8/ME10 were incubated in serum-free medium in the presence of 1 $\mu\text{Ci/ml}$ [^3H] thymidine for 24 hr and incorporated counts were determined as described in Example 8 herein. The means \pm SE of triplicate determinations from 7 separate experiments are shown.

Figure 10 illustrates the effect of antisense IGF IR cDNA transcription on VSMC proliferation. Growth of cells of clone CA9 (transfected with pAnti-IGF IR) and growth of cells of clone ME10 (transfected with vector alone) are compared. Cells were seeded at a density of 2×10^3 cells/well and maintained in culture medium containing 10% calf serum. The results shown are representative of an experiment which was repeated three times.

Detailed Description of the Invention

Type I insulin-like growth factor (IGF I) is a ubiquitous peptide that circulates at high levels in serum and is expressed in multiple tissues. It has a broad spectrum of effects including stimulation of embryonic and postnatal growth, cell growth and differentiation *in vitro*, various metabolic effects and participation in tissue regeneration. IGF I also participates in the undesirable proliferation of vascular smooth muscle cells, e.g., associated with post-angioplasty restenosis, restenosis after other vascular injury and with post-cardiac transplant atherosclerosis. The structure of IGF I is known, and the structure and recombinant expression of human IGF I are described in, e.g., U.S. Patent No. 5,324,639 (Brierly et al) and in Gritz and Davies (1983) *J. Gene* 25:179-188.

The effects of IGF I are mediated via binding to a specific heterotetrameric membrane receptor (IGF IR) that consists of two extracellular α -subunits and two membrane-spanning β -subunits, which receptor is widely distributed in mammalian tissues. Several studies have suggested that IGF I plays a unique role as an autocrine regulator of cellular function and that activation

of the IGF I/IGF IR loop is required for the entry of cells into S phase in response to a variety of growth stimuli. Ullrich et al (1986) *EMBO J* 5: describes the cDNA coding sequence (see also SEQ ID NO:10) and deduced amino acid sequence of human IGF IR.

5 SEQ ID NO:10 presents the human IGF IR cDNA sequence and predicted amino acid sequence. The N-termini of the α and the β subunits are at amino acids 1 and 711, respectively. Potential N-linked glycosylation sites are at amino acids 21-23, 72-74, 105-107, 214-216, 284-286, 397-399, 408-410, 504-506, 577-579, 10 592-594, 610-612, 726-728, 734-736, 870-872, 883-885 and 933-935. The transmembrane domain is at amino acids 906-929. A potential ATP binding site is at Gly residues at amino acid numbers 976, 978 and 981 and at Lys 1003. The putative precursor processing site is at 707-710, proteolytic cleavage here divides the precursor into the α and β subunits.

15 The involvement of IGF I and of the IGF IR in cardiovascular pathology has recently raised interest. *In vitro* data have established that IGF I is a smooth muscle cell mitogen (Pfeifle et al (1982) *Horm. Metab. Res.* 14:409-414; Clemmons, D.R. (1985) *Endocrinology* 117:77-83; Clemmons and Van Wyk (1985) *J. Clin. Invest.* 75:1914-1918) and several reports have documented that vascular smooth muscle cells (VSMC) express IGF I and its receptor *in vitro* and *in vivo* (See, e.g., Jialal et al (1985) *Endocrinology* 117:1222-1229; King et al. (1985) *J. Clin. Invest.* 75:1028-1036; Delafontaine et al (1991) *Hypertension* 18:742-747; Bornfeldt et al (1991) *Diabetologia* 34:307-313). In conditions in which there is excessive VSMC proliferation such as coarctation hypertension (Fath et al (1993) *Circul. Res.* 72:271-277), or after balloon angioplasty (Khorsandi et al (1992) *J. Clin. Invest.* 90:1926-1931), there is an increase in IGF I mRNA expression. Angiotensin II (Ang II), a vascular peptide that is a VSMC mitogen, transcriptionally regulates the IGF I gene in VSMC, and neutralization of extracellular IGF I abolishes Ang II-induced growth. Growth factors such as Ang II, platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) 35 increase IGF I receptors on VSMC, suggesting that upregulation

of IGF I receptors may play a role in growth factor induced mitogenic responses.

In general, the terminology used herein is standard, as understood by those of ordinary skill in the fields of molecular biology, biochemistry, cell biology and medicine. For added
5 clarity, certain terms are defined herein.

As used herein, an antisense oligonucleotide is one which has a nucleotide sequence that is complementary to a particular target nucleic acid molecule, usually a mRNA. In order to
10 effectively inhibit expression of the target mRNA, an antisense is preferably from about 14 nucleotides in length up to the entire target sequence. Oligonucleotides which are chemically synthesized are preferably about 18-22 nucleotides in length. When the antisense molecule is an antisense RNA, i.e., a
15 transcription product within a target cell, preferably the antisense RNA is at least about 100 nucleotides long, and may be as long as 1 Kb or larger. It is generally preferred that the nucleotide sequence of the antisense oligonucleotide is targeted to the region of the mRNA which includes the translation start
20 site.

As used herein, a sense oligonucleotide is one which has a sequence identical to at least a portion of a target nucleic acid molecule, usually a target mRNA. However, a sense oligonucleotide introduced into or expressed in a cell may
25 complex with a naturally occurring target nucleic acid molecule, or which significantly differs in overall nucleotide sequence (by more than about 45%) from a molecule of the same sense polarity.

In the instant context, an ATG-directed oligonucleotide can be sense or antisense relative to a target mRNA. ATG-directed
30 means that the oligonucleotide comprises the ATG or AUG translation start site in a sense oligonucleotide, or its complement CAT (or CAU in RNA) in the antisense oligonucleotide, and the limitations on length and possibly advantageous modifications described herein. An ATG-directed sense of
35 antisense RNA (a less than full length transcript specific for IGF IR or other growth factor receptors) similarly comprises the ATG (or AUG) translation start site or its CAT (or CAU in RNA)

complement, depending on whether it is sense or antisense, and with length limitations as described herein.

In the present context, a mismatch (or missense) antisense oligonucleotide is one which has an antisense nucleotide sequence in which there is significant mismatch with a complementary target nucleic acid molecule.

Where persistence of an oligonucleotide is useful (within a cell, in serum, in cell culture, in a therapeutic composition which has been applied to a target tissue), it is desirable to use an oligonucleotide comprising chemical modifications which contribute to chemical stability, resistance to enzymatic degradation and the like. Such chemically modified oligonucleotides include, but are not limited to, phosphorothioate (e.g., 2-O-methylphosphorothioate), phosphorodithioate, phosphotriester, methylphosphonate, phosphoramidite, α -anomer and phosphoroselenoate oligonucleotides. The ordinary skilled artisan can prepare such modified oligonucleotides using techniques and equipment well known and readily accessible to the art, and without the expense of undue experimentation (See, e.g., Helene and Toulme (1990) *Biochem. Biophys. Acta.* 1049:99-125 and references cited therein). The skilled artisan knows how to advantageously choose a particular modification for a particular application.

Phosphorothioate (sulfur-protected) oligonucleotides are relatively stable in serum, with about 50% remaining intact after 36 hours incubation, according to Shi et al (1993) *Circulation* 88:1190-1195. This reference describes uptake by and persistence in and inhibition of human smooth muscle cell proliferation by antisense phosphorothioate oligonucleotides directed against c-myc messenger RNA.

A non-naturally occurring recombinant nucleic acid molecule (DNA or RNA) is one which does not occur in nature, and it is thus distinguished from a chromosome, for example. A specific example of a recombinant DNA molecule of the present invention is a vector comprising a portion of an IGF IR sequences operably linked to expression control sequences such that transcriptional expression of at least a portion of the IGF IR sequences is

obtained. Those expression control sequences regulate and promote transcription, and they are not associated with IGF IR coding sequences in nature. Depending on the orientation of the IGF IR sequences relative to the expression control sequences, the transcription product will be sense or antisense, as understood in the art. In a specific embodiment, the transcript is an antisense RNA and is complementary to at least about 100 nucleotides of the native cellular IGF IR mRNA. Alternatively, the expression product is a sense RNA which is shorter than native cellular IGF IR mRNA, does not contain a full-length coding sequence, but which includes the region of the ATG translation start site. Preferably, the sense RNA is significantly shorter than the full length coding sequence, e.g., about 100 nucleotides in length. An additional embodiment is a vector in which a vertebrate IGF IR cDNA sequence with full length coding sequence (e.g., rat) has been inserted, operably linked to expression control sequences.

Vectors may be plasmids or virus vectors capable of being maintained in bacterial cells, yeast cells or in vertebrate, such as mammalian cell culture. These vectors may be designed for maintenance in a single-celled host (bacterium or yeast) and capable only of transient gene expression when introduced into a human, or other animal or capable of stable maintenance with either regulated (temporally and/or tissue-specific) or constitutive gene expression. The art knows how to choose vectors and expression control sequences to achieve a particular desired result from a number of known and readily accessible vectors and expression control sequences known to the art.

For wound and burn healing, bone repair and for collateral vascular development, transient expression is preferred. For antisense RNA expression in treatment and/or prevention of atherosclerosis, post balloon angioplasty restenosis and post transplant atherosclerosis and vascular smooth muscle cell hyperproliferative disorders, transient expression of antisense RNA is preferred. For uses where transient expression of an IGF IR-specific antisense RNA is appropriate, antisense oligonucleotides can be substituted. In applications of

stimulating IGF IR gene expression, such as in the production of leaner meat in, e.g., swine or beef cattle, prolonged expression of a relatively short (at least 14-20 nucleotides, but less than full length) sense ATG-directed IGF IR RNA is preferred.

5 The cDNA sequence for rat brain cell IGF IR was determined from several overlapping clones isolated as described in Example 1. Nucleotide sequence analysis of the cDNA resulted in deduction of the primary protein sequence of the rat IGF IR precursor (SEQ ID NOS: 8 and 9). The cDNA analyzed contains an
10 opening reading frame of 4113 nucleotides encoding a 1370 amino acid IGF IR precursor, with 45 bp of 5' untranslated and 538 bp of 3' untranslated flanking sequences. Based on the structure of the human IGF IR (Ullrich et al. (1986) supra), the first 30 encoded amino acids likely represent a signal peptide (amino
15 acids -30 to -1) with the putative start site of the mature α subunit being glutamate at position 1 (SEQ ID NO:9). Four arginine residues representing the putative cleavage site of the $\alpha\beta$ proreceptor precede the putative start site of the β subunit at Asp 712. The 17 amino acid hydrophobic sequence between
20 residues 908 and 924 is likely a transmembrane domain. Features consistent with kinase activity are located between amino acids 974 and 1230, including the tyrosine kinase class II signature pattern from Asp 1130 to Arg 1138 and a potential ATP-binding region from Leu 976 to Val 984. Lammers et al. (1989) *EMBO J.*
25 8:1369-1375 describes a recombinant vector for the expression of human IGF IR.

Analysis of the full-length rat IGF IR cDNA sequence (SEQ ID NO:8) revealed a high overall degree of similarity to the human sequence (Ullrich et al. (1986) supra). Within the protein
30 coding sequence there is approximately 89% sequence identity at the nucleotide level and about 98% sequence identity at the amino acid level. The deduced amino acid sequence is given in SEQ ID NO:9. The predicted rat α -chain comprises 707 amino acids (compared with 706 for the human) with an 89% and 98% similarity
35 to the human IGF IR α -chain at the nucleotide and amino acid levels, respectively. There are 32 cysteine residues within the α -chain, including 24 cysteine residues in a cysteine-rich region

extending from Cys 148 to Cys 302. These are perfectly conserved between the rat and human receptors, consistent with the proposed important function of disulfide bridging in assembly of the α_2 - β_2 mature receptor. A putative cleavage site formed by four arginines (contrasting with the Arg Lys Arg Arg sequence of the human receptor) after residue 707 precedes the putative start site of the β subunit at Asp. Like the α -chain, the β -chain is highly conserved between rat and human with 90% and 98% similarity at nucleotide and amino acid levels. A 17 amino acid hydrophobic sequence (residues 908 to 924) likely represents the transmembrane domain, and differs from the corresponding human sequence by the substitution of an isoleucine for valine at position 915. The highly conserved tyrosine kinase domain contains a protein kinase ATP binding signature (Leu 976 to Val 1084), a tyrosine kinase specific active-site signature (Phe 1102 to Val 1114), and a receptor tyrosine kinase class II signature (Asp 1130 to Arg 1138). These sites are perfectly conserved between rat and human, as are the positions of cysteines within the rat and human β -chains. Like the human receptor, there are 17 potential N-glycosylation sites in the rat receptor. In contrast to the coding sequence, the 3'-untranslated region of the rat IGF IR has a lower (72%) degree of similarity to the human sequence.

As shown in Figure 1, incubation of VSMC with increasing concentrations of Antisense (AS) oligonucleotides (ODN) (SEQ ID NO:1) for 48 hr in serum-free medium decreased [3 H]-thymidine incorporation (69 \pm 7.6% decrease with a concentration of 10 μ M, as compared with serum-free medium alone). In addition, the mitogenic response to 10% calf serum was inhibited (56% decrease with a concentration of 10 μ M, as compared with 10% calf serum alone). There was no significant change in either basal or serum-induced DNA synthesis rates in cells exposed to mismatch antisense oligonucleotides (SEQ ID NO:3). Surprisingly, incubation of VSMC in serum-free medium for 48 hr with sense (S) oligonucleotide (SEQ ID NO:2) significantly increased [3 H]-thymidine incorporation - 145% increase above serum-free medium alone, with a concentration of 10 μ M S oligonucleotides.

Furthermore, the mitogenic response to 10% calf serum was maintained in cells pre-exposed to sense ATG-directed oligonucleotide. Thus the incorporation of [³H]-thymidine into cells exposed to 10 μ M S ODN (SEQ ID NO:2) for 48 hr and then to 10% calf serum was 61% greater than that of cells exposed to serum-free medium alone for 48 hr and subsequently to 10% calf serum. In order to establish correlations between DNA labeling indices and cell proliferation, cell counts were performed. Treatment of VSMC with 10 μ M AS oligonucleotide (SEQ ID NO:1) for 96 hr in the presence of 10% calf serum reduced cell number by 58% compared with cells maintained in 10% calf serum alone (Fig. 2). M ATG-directed oligonucleotides (SEQ ID NO:3) had no effect on cell proliferation, whereas ATG-directed S oligonucleotide (SEQ ID NO:2) increased cell number (30% increase with 10 μ M S ODN over 10% calf serum alone).

To assess the effects of oligonucleotides on IGF I-induced growth (mitogenic) response, cells were incubated in 10% calf serum \pm oligonucleotides (ODNs) for 48 hr and then exposed to serum-free medium in the presence or absence of increasing concentrations of IGF I (Fig. 3). IGF I caused a dose-dependent increase in [³H]-thymidine incorporation that was markedly blunted by pre-incubation of cells with ATG-directed AS ODNs. Thus, [³H]-thymidine incorporation stimulated by 20 ng/ml IGF I was reduced by 62% in AS-treated cells, compared with control. Exposure of cells to 5 μ M S ODN increased [³H]-thymidine incorporation basally (187% above control) and in response to IGF I (67% increase above control following incubation with 20 ng/ml IGF I).

A neutralizing antibody specific for IGF I inhibits ang II-induced DNA synthesis in VSMC (Delafontaine et al. (1991) *J. Biol. Chem.* 268:16866-16870). These findings suggested that activation of the IGF IR was required for Ang II-induced growth (mitogenic) responses in VSMC. We therefore assessed the effects of ODNs on ang II-induced [³H]-thymidine incorporation. As shown in Figure 4, Ang II caused a dose-dependent increase in DNA synthesis that was unaltered by pre-exposure of cells to ATG-directed M ODN (SEQ ID NO:3) but was markedly reduced following

pre-exposure to ATG-directed AS ODN (SEQ ID NO:1). Thus, there was a 75% reduction in [³H]-thymidine incorporation in response to 100 nM ang II in AS-treated cells, compared with control. S-treated cells (SEQ ID NO:2) had a marked increase in [³H]-thymidine incorporation basally (297% above control). Ang II had no additive mitogenic effect on S-treated cells.

To establish correlations between the observed effects of ODNs on growth and IGF IR binding parameters, radioligand binding experiments were performed. Exposure of VSMC to 5 μ M ATG-directed AS ODN (SEQ ID NO:1) for 48 hr in serum-free medium reduced IGF IR number by 56%, compared with control (Fig. 5A). M ODN (SEQ ID NO:3) had no effect on IGF I binding capacity, while S ODN (5 μ M) (SEQ ID NO:2) increased IGF IR number by 39%. Exposure of VSMC to ODNs in the presence of 10% calf serum produced similar results (Fig. 5B). Thus, 5 μ M AS ODN (SEQ ID NO:1) reduced IGF IR by 57% and 5 μ M S ODN (SEQ ID NO:2) increased IGF IR by 69%, compared with 10% calf serum alone. There was no effect of IGF IR ODNs on IGF I binding-affinity. To further confirm the specificity of these findings, [¹²⁵I]-sar¹¹-ile⁸ang II binding studies were performed on cells exposed to IGF IR ODNs. AS, S, and M ODNs (SEQ ID NOS:1, 2 and 3, respectively) had no effect on ang II receptor number (Fig. 5C) or affinity.

To determine whether the observed effects of S ODN (SEQ ID NO:2) on VSMC IGF IR number and growth responses were site-specific, experiments were conducted using AS and S oligomers targeting a sequence starting at 109 nucleotides downstream of the ATG. As shown in Fig. 6A, AS ODNs of SEQ ID NO:4 produced the expected reduction in [³H]-thymidine incorporation, basally and in response to 10% calf serum. However, in marked contrast to results obtained using S ODNs targeting the ATG site (i.e., SEQ ID NO: 2), S ODNs targeting the sequence about 100 bases 3' to the ATG site (SEQ ID NO:5) did not significantly alter DNA synthesis, either basally or in response to serum. Furthermore, radioligand binding studies established that these ODNs had the corresponding effects on IGF IR number. Thus, exposure of VSMC to 5 μ M SEQ ID NO:4 AS ODN in the presence of serum-free medium or 10% calf serum reduced IGF IR number by 28% and 38%

respectively, while there was no significant effect of SEQ ID NO:5 S ODNs on IGF IR number (Fig.6B).

To determine whether effects of ODNs on IGF IR number correlated with changes in steady-state levels of IGF IR mRNA, solution hybridization/RNase protection assays were performed (Fig. 7). AS oligonucleotides complementary to IGF IR mRNA decreased IGF IR mRNA levels (~40% decreases with 5 μ M ODNs directed at ATG and non-ATG sites (SEQ ID NOS:1 and 4, respectively). S ODNs (SEQ ID NO:2) targeting the ATG site did not significantly increase IGF IR mRNA levels, while S ODNs (SEQ ID NO:5) targeting the sequence about 100 bases 3' to the ATG site did not significantly affect IGF IR mRNA levels.

To further address the function of the IGF IR in VSMC growth, an antisense IGF IR construct was stably transfected into primary rat aortic smooth muscle cells (RASM). Our results show that VSMC expressing this antisense transcript have a decrease in endogenous IGF IR mRNA levels, particularly at confluence and post-confluence. The reduction in IGF IR mRNA levels in post-confluent cells of ~57% correlates well with the 51% decrease in surface IGF IR binding sites. DNA synthesis in antisense-expressing cells was decreased by 55%, indicating that a reduction in IGF IR number/cell inhibited VSMC growth. Furthermore, antisense-expressing cells had a 59% decrease in IGF I-stimulated DNA analysis. These findings were substantiated by growth curves which showed a marked reduction in the proliferation of CA9 cells maintained in 10% serum. Interestingly, there was only a small decrease in IGF IR mRNA levels in antisense-expressing cells at preconfluence, correlating with a 35% reduction in IGF IR number. The lesser reduction in IGF IR mRNA in preconfluent cells may relate to the fact that preconfluent control cells have approximately 1.5-fold higher basal IGF IR mRNA levels than post-confluent cells. The greater reduction in IGF IR at confluency in antisense cells correlates with a marked reduction in cellular growth rates. Because serum contains a variety of growth factors, these results suggest that the IGF IR participates in growth responses to multiple agonists.

In order to detect antisense IGF IR mRNA, northern hybridization analysis was performed using a ^{32}P -labeled sense riboprobe generated using T7 polymerase transcription of a partial IGF IR sequence (nucleotides +560 to +680). CA9 cells (which express antisense RNA from a vector, see Example 3 and 5), at both preconfluence and post-confluence expressed abundant amounts of the 1.0 kb antisense transcript, while this transcript was undetectable in control clone ME10 (containing vector without IGF IR-related insert). To determine the effect of antisense IGF IR mRNA on endogenous IGF IR mRNA levels, solution hybridization/RNase protection assays were performed using an antisense IGF IR riboprobe, co-hybridized with an antisense GAPDH riboprobe as an internal control. There was a small reduction in IGF IR mRNA levels in preconfluent cells and marked reduction in post-confluent cells. Densitometric analysis of IGF IR mRNA levels (corrected for the GAPDH signal) indicated that IGF IR antisense-expressing CA9 cells, compared with ME10 cells, had a reduction in IGF IR mRNA of $18 \pm 4.5\%$ (mean \pm SE, $n=2$) at preconfluence, and of $57 \pm 4.8\%$ (mean \pm SE, $n=3$) at post-confluence.

In order to assess the effect of antisense IGF IR cDNA transcription on IGF I binding, ^{125}I -IGF I radioligand binding studies were performed with CA9 cells and control ME8 and ME10 cells, all at preconfluence and at confluence/up to 2 days post-confluence. Results from scatchard analysis of displacement curves indicated that there was a 35% reduction in IGF IR number/cell in preconfluent CA9 cells, as compared with preconfluent control ME8/ME10 cells (CA9, 44 ± 1.0 fmol/ 10^5 cells; ME8 and ME10, 68 ± 2 fmol/ 10^5 cells, $n=2$). There was a 51% reduction in IGF IR number in confluent CA9 cells, as compared with confluent ME8 or ME10 cells. There was no significant difference in IGF IR binding affinity between CA9 cells ($K_d = 5.6 \pm 1.0$ nM, $n = 10$) and ME8/ME10 cells ($K_d = 6.7 \pm 1.5$ nM, $n = 10$), either at preconfluency or confluency.

To determine whether the reduction in IGF IR density on CA9 cells altered DNA synthesis, [^3H]thymidine incorporation was measured in CA9 cells and in control ME10 cells. There was a 55%

reduction in [³H]thymidine uptake in CA9 cells as compared with ME10 cells. To determine whether the mitogenic response to IGF I was altered by reduction in IGF IR density, DNA synthesis was measured in confluent cells exposed to IGF I (10 ng/ml, 24 hr.).

5 There was a 59% reduction in IGF I-stimulated [³H]thymidine incorporation in CA9 cells (585 ± 198 dpm/ 10^4 cells, $n = 3$) as compared with ME8/ME10 cells (1428 ± 92 dpm/ 10^4 cells, $n = 2$).

To further characterize the effect of IGF IR antisense targeting on cell proliferation, growth curves were performed

10 using CA9 and ME10 cells maintained in the presence of 10% calf serum. There was a marked inhibition of the proliferative response of CA9 cells. The antiproliferative effect became evident at day 7, as the cells reached confluence. The mean of results from three independent experiments indicated that there

15 was a $60 \pm 3.4\%$ reduction in cell number at day 7 after plating in CA9 cells, as compared with ME10 cells.

Anti-IGF I antiserum has been shown to inhibit PDGF-mediated growth of VSMC (Clemmons and Van Wyk (1985) supra).

20 Delafontaine and Lou (1993) supra showed that Ang II transcriptionally regulated IGF I expression in VSMC and that anti-IGF I antibody inhibits Ang II induced DNA synthesis. In neuroepithelial cells FGF-mediated proliferation is dependent on IGF I (Drago et al. (1991) *Proc. Nat'l Acad. Sci. USA* 88:2199-2203).

25 There is some experimental suggestion of the importance of IGF IR number/cell in cellular growth responses. Antisense targeting of the IGF I/IGF IR system has provided evidence for its role in normal organ growth (Wada et al. (1993) *Proc. Nat'l. Acad. Sci. USA* 90:10360-10364) and in tumorigenesis (Trojan et al. (1992) *Proc. Nat'l. Acad. Sci. USA* 89:4874-4878).

30 Furthermore, overexpression of the IGF IR has been shown to induce transformation of NIH/3T3 cells *in vitro*, allowing these cells to form tumors in nude mice (Kaleko et al. (1990) *Mol. Cell. Biol.* 10:464-473). The results presented herein, taken

35 with what was known, indicate that IGF IR plays a crucial role in VSMC proliferative responses and provides the basis for antisense methods to down-regulate IGF IR gene expression to

retard VSMC growth and proliferate where undesirable, e.g., in treatments to prevent post angioplasty restenosis, restenosis after vascular injury or post-heart-transplant atherosclerosis in the coronary arteries and vascular complications of hypertension including proliferative retinopathy, cerebrovascular disease and hypertensive nephropathy. And, on the contrary, ATG-directed sense oligonucleotides and methods using same allow for stimulation of cell growth and proliferation where desirable. Such situations where increased IGF IR number/cell is desirable include healing of broken bones, burns and other wounds, nerve regeneration after injury, and angiogenesis in tissue affected by peripheral vascular disease or by myocardial infarction.

The foregoing results clearly demonstrate that IGF IR density on vascular smooth muscle cells is an important determinant of their growth responses to serum and to angiotensin II. Thus, targeting of the IGF IR mRNA with antisense ODNs complementary thereto, as exemplified with either a sequence spanning the translation initiation codon or the sequence beginning about 109 bp downstream thereof, reduces IGF IR mRNA levels and IGF IR number without altering IGF IR binding-affinity (K_d). This inhibition of IGF-I Receptor expression results in a decrease in basal [3 H]-thymidine incorporation as well as in the mitogenic response to the addition of 10% serum. The antiproliferative effect of the antisense oligonucleotides is reflected in the reduction of cell counts after 96 hr incubation of VSMC with increasing doses of AS ODN in the presence of 10% calf serum. Because serum contains a variety of growth factors, DNA synthesis rates in response to IGF I were measured; [3 H]-thymidine incorporation in response to IGF I was inhibited in a dose-dependent manner by antisense oligonucleotides. The mitogenic response to ang II was also inhibited by antisense oligonucleotides complementary to IGF IR mRNA, confirming the crucial role of the autocrine IGF I ligand-receptor system in ang II-induced growth of VSMC. It was previously demonstrated that an anti-IGF I antibody inhibits ang II-induced mitogenesis in VSMC (Delafontaine et al. (1993) supra). The effect of IGF IR antisense oligonucleotides on ang II mitogenesis was specific to

IGF IR gene expression, in that ang II binding capacity was not altered.

5 In contrast to findings with antisense oligonucleotides, an ATG-directed mismatched antisense oligonucleotide (M, SEQ ID NO:3) (based on the antisense sequence of SEQ ID NO:1 but with a 9 of 20 bp mismatch) had no effect on IGF I receptor number, [3H]-thymidine incorporation (basally or in response to serum), cell proliferation and the mitogenic response to ang II. These data confirm the specificity of the molecular effects of IGF IR antisense oligonucleotides.

10 Unexpectedly, exposure of VSMC to a sense oligonucleotide corresponding to sequence spanning the initiation codon of the IGF IR (e.g., S, SEQ ID NO:2) resulted in an increase in IGF IR number per cell, associated with an increase in basal [3H]-thymidine incorporation (in serum-free medium) as well as in the presence of 10% serum. This was reflected in an increase in cell proliferation. The increase in IGF IR density induced by S ODN of SEQ ID NO:2 was associated with an increase in the mitogenic response to IGF I. Interestingly, however, ang II had no additive stimulatory effect on [3H]-thymidine incorporation following pre-incubation of cells with S ODN (SEQ ID NO:2). This suggests that at a certain level of activation of the IGF IR, angiotensin II does not further stimulate mitogenesis.

25 Molecular mechanisms responsible for ATG-directed S oligonucleotide-mediated increases in IGF IR number have not yet been elucidated. The increase is associated with an increase in IGF IR mRNA levels and is sequence-specific. Thus, although an AS ODN targeting a sequence at bp + 109 (relative to ATG) (SEQ ID NO:4) reduced IGF receptor number and inhibited VSMC growth, the corresponding sense oligonucleotide targeting this site (SEQ ID NO:5) had no effect on either IGF IR number or VSMC growth. Without wishing to be bound by any particular theory, one potential explanation for S ODN-mediated upregulation of IGF IR is the presence of an endogenous IGF IR antisense mRNA species in VSMC, which is effectively "neutralized" by the administered sense oligonucleotide. However, reports of naturally occurring antisense RNAs in eukaryotic systems are rare (Inouye, M. (1988)

Gene 72:25-34; Colman, A. (1990) *J. Cell. Science* 97:399-409; Helene et al. (1990) *Biochem. Biophys. Acta.* 1049:99-125).

Alternatively, and again without wishing to be bound by any particular theory, the S mRNA may bind to its complementary DNA sequence through Watson-Crick bonding or through triple helix formation and increase transcription by facilitating the opening of the DNA helix. Indeed, local openings of the double helix have been shown to correlate with DNA template activity, and clearly, epigenetic RNA molecules are capable of stabilizing these openings (Reiss et al. (1991) *Cancer Research* 51:5997-6000. A third non-binding potential explanation is that the ATG-directed sense oligonucleotide may bind with a protein factor to effect positive regulation of IGF IR gene expression.

These data indicating a key regulatory role of the IGF IR in VSMC growth are consistent with other observations in fibroblasts. In BALB/c3T3 fibroblasts, Epidermal Growth Factor (EGF) upregulates IGF I expression; and secretion and targeting of the IGF IR through use of antisense oligonucleotides inhibits EGF-induced growth (Pietrzkowski et al. (1992) *Mol. Cell. Biol.* 12:3883-3889). Furthermore, in BALB/c3T3 cells overexpressing IGF I and IGF IR, IGF I-mediated growth occurs independently of the EGF and PDGF receptors (Pietrzkowski et al. (1992) *Cell Growth Differ.* 3:199-205). Constitutive expression of c-myb in 3T3 cells has been shown to upregulate IGF I and IGF IR expression, thereby abrogating the requirement of these cells for exogenous IGF I and suggesting that IGF IR activation may be important mechanically in the effect of c-myb on cell proliferation (Reiss et al. (1991) *supra*; Travali et al. (1991) *Mol. Cell. Biol.* 11:731-736).

Moreover, in SV40 T antigen-transformed BALB/c3T3 cells, antisense targeting of the IGF I receptor has been shown to inhibit growth (Porcu et al. (1992) *Mol. Cell. Biol.* 12:5069-5077). The results provided herein are strong evidence that IGF IR density is an important factor in mediating IGF I and serum-induced growth responses in vascular smooth muscle cells. Thus, even in the presence of high concentrations of IGF I, the mitogenic response of antisense-treated cells is markedly

blunted. The data suggest that IGF I, acting through its tyrosine-kinase receptor, may serve as an important co-factor or intermediary in the growth response to a variety of agonists. This is consistent with its known effects at the G₁/S phase of the cell cycle (Stiles et al. (1979) *Proc. Natl. Acad. Sci USA* 76:1279-1283).

Manipulation of IGF IR density on vascular smooth muscle cells markedly alters the growth responses of these cells to IGF I, ang II and serum. Receptor availability has marked effects on ligand responsiveness. This ligand-receptor system is crucial for the control of VSMC growth in vitro and in vivo. Furthermore; the surprising observation that a sense oligonucleotide targeting of the ATG site of the IGF IR message upregulates IGF IR identifies a novel use for synthetic oligonucleotides in the regulation of gene expression.

As demonstrated herein, the density of IGF IR can be down-regulated via antisense oligonucleotides or antisense RNAs complementary to at least a portion of the IGF IR mRNA (≥ 14 contiguous nucleotides for oligonucleotides, at least 100 nucleotides for intracellularly transcribed antisense RNAs). Antisense oligonucleotides and antisense RNAs inhibit vascular smooth muscle cells in vitro and have the same effect in vivo.

Generally, when oligonucleotides are used, they are in a modified form which increases persistence in cells and/or in the extracellular milieu. An exemplified modified modification is phosphorothioate oligonucleotides. ATG-directed sense oligonucleotides (e.g., phosphorothioate oligonucleotides) specific for IGF IR mRNA can be delivered when the proliferation of vascular smooth muscle cells, or when collateral blood vessel development is desired. For delivery of oligonucleotides or recombinant vectors to the inner surfaces of an artery, a catheter can be used or a porous balloon generally similar to that used in balloon angioplasty can be used (See, e.g., Shi et al. (1994) *Circulation* 90:944-951; Guzman et al. (1993) *Circulation* 88:2838-2848; Ohno et al (1994) *Science* 265:781-784).

For wound, burn and bone healing applications or where collateral blood vessel development, angiogenesis or nerve

regeneration is desired, ATG-directed, IGF IR-specific oligonucleotides, preferably modified for resistance to degradation (e.g., in phosphorothioate form), are formulated into pharmaceutical compositions.

5 For delivery to wounded or burned tissue or damaged bone, pharmaceutical preparations of IGF IR-specific, ATG-directed sense oligonucleotides, preferably a modified oligonucleotide such as a phosphorothioate oligonucleotide can be formulated, in a therapeutically effective amount, with a suitable carrier for
10 topical application or other local application such as introduction via catheter. An adenovirus vector capable of expressing an IGF IR-specific, ATG-directed sense RNA can also be formulated into a healing-promoting preparation. Preferably such a vector is replication-deficient.

15 Where inhibition of IGF IR receptor gene expression is desired, an IGF IR-specific antisense oligonucleotide (such as a phosphorothioate oligonucleotide) or an antisense-expressing recombinant vector can be incorporated. In a situation where angiogenesis is advantageously inhibited an IGF IR-specific
20 antisense-expressing recombinant vector or oligonucleotide preparation can be directed to a target tissue such as a solid tumor using a means known to the art. One example is the delivery to prostate tumor or other tumor bearing androgen receptors using the methods and compositions disclosed in USSN
25 08/283,238 filed July 29, 1994, incorporated by reference herein in its entirety.

Suitable excipients for therapeutic/pharmaceutical compositions are described in, e.g., Remington's Pharmaceutical Sciences, by E.W. Martin. Other components such as stabilizers
30 including chelating agents, e.g., EDTA, sugars, sugar alcohols, antioxidants and nonionic surfactants such as polyethylene glycol or block co-polymers of polyethylene and polypropylene glycol may be added.

Therapeutic oligonucleotide (or vector) compositions of the
35 present invention contain a therapeutically effective dose of the active ingredient in a pharmaceutically acceptable carrier. The dose, carrier, route and schedule of administration of choice

depend among other factors, upon each other, the condition to be treated and the patient's species. These factors are readily determined and monitored by the treating physician or veterinarian during the course of therapy.

5 All references cited herein are hereby incorporated by reference in their entirety.

Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction
10 endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York;
15 Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold spring Harbor Laboratory, Cold Spring Harbor, New York, Old Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender
20 (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

30 The foregoing discussion and the following examples illustrate but are not intended to limit the invention. The skilled artisan will understand that alternative methods and compositions may be used to implement the invention.

EXAMPLESExample 1 Cloning of IGF I Receptor cDNA

Total RNA was isolated from rat aortic smooth muscle cells (RASM) and following first strand cDNA synthesis a 1.1 kb cDNA probe was generated using Polymerase Chain Reaction, PCR. Primers D1 (5'-CCCCAAATAAAAGGAATG-3') (SEQ ID NO:6) and U1 (5'-AGCAGATTGCCCTTC-3') (SEQ ID NO:7) were based on the sequence of Werner et al. (1989) *Proc. Nat'l Acad. Sci. USA* 86: 7451-7455. The PCR product was directly cloned into the pCRII vector (Invitrogen Inc., San Diego, CA), and the resulting clone (p118) was sequenced and used to screen a λ gt10 cDNA library from adult male Sprague-Dawley rat brain (Clontech, Palo Alto, CA). 1.2×10^6 clones were screened, and 30 of 80 positive clones were analyzed by PCR using a λ gt10 arm universal primer and an IGF IR internal primer to determine insert size. After two additional cycles of screening, clones containing longer inserts were isolated, purified and the library was rescreened using these clones and a 3' specific probe (from 2887-2906 bases downstream of the IGF IR translation start site). EcoRI inserts of purified phage clones were subcloned into pGEM3 (Promega Corp., Madison, WI) and sequenced using the sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, OH). Ambiguities were resolved by resequencing using a modified 40% formamide gel (J.T. Baker, Inc., Phillipsburg, NJ). DNA sequence data was assembled from nine overlapping clones and analyzed using the Genetics Computer Group program (Madison, WI). The deduced amino acid sequence data was analyzed using PC Gene (IntelliGenetics, Inc., Mountain View, CA). A full-length rat cDNA clone for IGF IR was also isolated.

Example 2 Oligonucleotide Synthesis

Phosphorothioate 20-mer oligonucleotides (ODNs) synthesized using an Applied Biosystems instrument (Applied Biosystems, Foster City, CA) by the Microchemical Facility, Emory University, HPLC purified, resuspended in TE (10 mM Tris, 1 mM EDTA; pH 8.0), and quantified by spectrophotometry. Antisense (AS), sense (S) and mismatch (M) ODNs targeting a sequence starting 2 nucleotides

5' to (upstream of) the ATG site of rat IGF IR cDNA were respectively; AS, 5'-TCCGGAGCCAGACTTCATTC-3' (SEQ ID NO:1), S, 5'-GAATGAAGTCTGGCTCCGGA-3' (SEQ ID NO:2); M, 5'-AGCGGTCCCACTCTTGTGTG-3' (SEQ ID NO:3). ODNs targeting a sequence starting 109 bases downstream of the ATG were AS, 5'-CAGCTGCTGATAGTCGTTGC (SEQ ID NO:4) and S, 5'-GCAACGACTATCAGCAGCTG-3' (SEQ ID NO:5). Oligonucleotides were filter-sterilized and used at a concentration of 0.1-10 μ M.

Example 3 Construction of Antisense Rat IGF IR Vector.

10 A diagrammatic representation of the vector assembly is shown in Figure 8. Briefly, the rat IGF IR cDNA clone p118 was digested by XhoI and KpnI and the 0.8 kb restriction fragment (from nucleotide +277 to +1086 relative to ATG) was ligated in an antisense orientation into pCEP4 (Invitrogen). (See also SEQ ID NO:8.) Transcription of the antisense IGF IR cDNA is under control of the cytomegalovirus immediate early gene enhancer/promoter, and yields a 1.0 kb antisense transcript (including linker and polyadenylated tail). This vector contains two Epstein Barr virus (EBV) derived genes namely, the EBV ori-P (origin of replication) and the EBV-nuclear antigen 1, which allow episomal replication. The SV40 poly(A) tail provides a termination signal for transcription, and a hygromycin resistance gene allows selection of transfectants.

Example 4 Cell Culture

25 Vascular smooth muscle cells (VSMC) were isolated from rat thoracic aorta by enzymatic dissociation as described by Gunther et al. (1982) *J. Cell. Biol.* 92:289-298. They were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (CS), 2 mM glutamine, 100 units/ml penicillin and 100 μ g/ml streptomycin, and passaged twice a week by harvesting with trypsin-versene and seeding at a 1:8 ratio in 75 cm² flasks.

For oligonucleotide experiments, cells between passage levels 5 and 15 were seeded into 100-mm, 24-well or 48-well polystyrene cluster culture dishes. For certain experiments cells were quiesced by exposure to defined serum-free medium

(SFM) containing DMEM and Ham's F12 (1:1) supplemented with transferrin (5 $\mu\text{g/ml}$), insulin (5×10^{-7} M), ascorbate (0.2 mM), glutamine and antibiotics.

Example 5 Cell Transfection and Selection

5 Vascular smooth muscle cells were isolated and cultured as described in Example 4 hereinabove. For transfections, ~10% confluent (passage number five) cells in 100 mm dishes were incubated in the presence of 10 μg of calcium phosphate precipitate of carrier DNA (pRSV-CAT, Invitrogen) and the plasmid
10 pAnti-IGF IR in a 1:2 ratio for six hours as described by Chen and Okayama (1988) *Bio Techniques* 6:632-638. Cells were grown in hygromycin-free medium for 72 hours and then selected in the presence of hygromycin (Sigma) at a concentration of 100 $\mu\text{g/ml}$. This concentration of antibiotic was determined to effectively
15 kill VSMC. Selection medium was replaced every three days. After six weeks, hygromycin resistant colonies were isolated, cloned into 24 well plates and subsequently expanded into 12 and 6 well tissue culture plates.

20 Eleven clones stably transfected with pAnti-IGF IR and 25 clones transfected with vector alone were selected by hygromycin resistance and amplified. The presence of the vector sequence in transfected cells was confirmed by Southern blotting, and northern hybridization analysis was used to screen for presence of antisense IGF IR mRNA. Two clones transfected with pAnti-IGF
25 IR exhibited high level expression of the 1 kb antisense IGF IR mRNA, and of these, clone CA9 was selected for further study. Two clones transfected with vector alone (ME8 and ME10) were selected as controls for further characterization.

Example 6 Measurement of DNA synthesis

30 To measure effects of ODNs on the growth response to 10% serum, VSMC were grown to 80% confluence in 48-well plates, serum-deprived for 48 hr in the presence or absence of ODNs (0.1-10 μM) and then exposed to fresh SFM in the absence of ODNs, or to 10% serum, for 24 hr. For cells transfected to transcribe
35 oligonucleotides, 1 $\mu\text{Ci/ml}$ [^3H]-thymidine was included during the

last 24 hr. Cells were then washed 3x with ice-cold phosphate buffered saline, incubated on ice for 15 min with 10% trichloroacetic acid (TCA), filtered with two washes in ice-cold 95% ethanol, air dried, and then insoluble radioactivity was
5 extracted with 0.4 N NaOH and counted by liquid scintillation spectrophotometry after neutralization. All experiments were performed in triplicate.

In order to measure the effects of ODNs on the mitogenic response to IGF I, VSMC were grown to 50% confluence and then
10 incubated in DMEM with 10% CS alone, or in the presence of 5 μ M AS or 5 μ M S ODN for 48 hr. Cells were then washed in serum-free medium (SFM) and incubated in SFM \pm IGF I (1-100 ng/ml) for 24 hr. 1 μ Ci/ml [3 H]-thymidine was present during the latter 24 hr., and TCA-precipitable counts were determined as described
15 above.

To measure the effects of ODNs on the mitogenic response to ang II, VSMC were exposed to AS or S ODNs (5 μ M) for 48 hr in the presence of 10% CS. Cells were then washed in SFM and exposed to SFM \pm ang II (1-1000 nM) for 24 hr. At 24 hr cells were
20 pulsed with [3 H]-thymidine (1 μ Ci/ml) for 12 hr in the continued presence of ang II. TCA-precipitable counts were then determined as described above.

Example 7 Growth Assay

VSMC were grown to 50% confluence in 48-well plates and then
25 exposed to SFM alone, 10% CS alone or 10% CS and increasing concentrations (0.1-10 μ M) of AS, S, or M ODNs. Medium and ODNs were replaced at 48 hr. At 96 hr cells were trypsinized and counted. To analyze growth curves on cells with transcribed antisense IGF IR oligonucleotides, transfected cells were split
30 into 24-well culture plates at a density of 2,000 cells per well, and grown in the presence of 10% calf serum and 100 μ g/ml hygromycin. Cells in duplicate wells were washed with PBS, harvested with PBS/20 mM EDTA and counted manually. Culture medium replacement and cell counts were performed every two days.

Example 8 Binding Assays

To determine the effect of ODNs on IGF IR number per cell and binding-affinity, VSMC were grown to 80% confluence in 24-well plates, and then exposed to SFM alone or in the presence of AS, S, or M ODNs (5 μ M) for 48 hr. For some experiments cells were grown to 50% confluence and then exposed to 10% CS alone or in the presence of AS or S ODNs (1,5 μ M) for 48 hr. Cells were washed in PBS and incubated at 37°C for 60 min. in binding buffer (20 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 10 mM NaHCO₃, 1.3 mM CaCl₂, 1.2 mM KH₂PO₄, 0.25% bovine serum albumin, pH 7.4) to allow dissociation of cell-bound IGF I. Cells were then rewashed and then incubated in the presence of 10⁻¹⁰ M ¹²⁵I-IGF I and increasing (0-10⁻⁷ M) concentrations of unlabeled IGF I for 90 min at room temperature. Cells were washed in ice-cold binding-buffer and solubilized in 2N NaOH before counting using an automated gamma counter with about 80% efficiency. All assays were performed in duplicate for each experimental point. Data were analyzed (scatchard analysis) using the LIGAND program. For measurement of Ang II binding, cells were incubated for 48 hr in 10% CS alone or in the presence of ATG-directed antisense (AS), sense (S) or missense (M) oligonucleotides (5 μ M). Binding studies were performed essentially as described above, except 10⁻¹⁰ M [¹²⁵I-Sar¹-Ile⁸]-ang II and 0-10⁻⁵ M unlabelled ang II were used for displacement curves.

For studies of the effects of transcribed IGF IR oligonucleotides, confluent and up to two days post-confluent 24-well culture plates of VSMC transfected with vector alone (ME8, ME10) or with pAnti-IGF IR (C9) were assayed and the data were analyzed as for the oligonucleotide-treated cells.

Example 9 Solution hybridization/RNase Protection Assay

For determination of IGF II mRNA levels a 203 bp EcoRI and KpnI rat IGF IR cDNA fragment was ligated into pGEM3 (Promega Corp., Madison, WI). The subclone p26K was linearized with EcoRI to allow generation of antisense RNA probe using SP6 RNA polymerase. Solution hybridization assays were performed by hybridizing 30 μ g of total RNA with 5 x 10⁵ cpm [³²P]-UTP labelled

AS IGF IR riboprobe and co-hybridizing with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe as previously described (Delafontaine et al. (1991) *Hypertension* 19:693-699; Delafontaine et al. (1992) *Hypertension* 18:742-747; Delafontaine and Lou (1993) *J. Biol. Chem.* 268:16866-16870). In brief, 30 μ g of total RNA was hybridized with 5×10^5 cpm of [32 P]-UTP labeled antisense IGF IR riboprobe and co-hybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe (Fort et al. (1985) *Nucl. Acids Res.* 13:1431-1442) in a solution containing 80% deionized formamide, 40 mM PIPES, pH 6.4, 0.4 M NaCl, 1 mM EDTA. After hybridization at 42°C and RNase digestion using 40 μ g/ml RNase and 100 units/ml RNase T1, samples were proteinase K treated, phenol extracted, and analyzed by 6% polyacrylamide/8 M urea denaturing gel electrophoresis. Full-length IGF IR probe is 251 bp, and protected fragment is 195 bp. The GAPDH protected fragment is 156 bp. Autoradiography was performed with 1-3 days exposure, and protected bands were quantitated by two-dimensional laser densitometry.

Example 10 Northern Hybridization Analysis

Transfected VSMC were studied at preconfluence (~80% confluent) and at 2 days post-confluence. Total RNA was prepared from stably transfected VSMC using the TRI-reagent method (Molecular Research Center, Inc., Cincinnati, OH), subjected to 1.2% agarose-formaldehyde gel electrophoresis, and transferred to a nylon membrane (Genescreen Plus, New England Nuclear, Boston, MA). The filter was stained with methylene blue to monitor RNA loading and RNA integrity. A 32 P-labeled rat IGF IR sense RNA probe (120 bp, nucleotides +560 to +680) was generated by T7 polymerase transcription of the rat IGR IR cDNA clone p522, after linearizing with PvuII. Hybridization conditions were as recommended by Promega (Madison, WI) i.e., 7% PEG 8000, 7% SDS, 50% formamide, 0.25 M NaCl, 0.25 M NaPO₄, pH 7.2, 100 μ g/ml herring sperm DNA and 100 μ g/ml yeast tRNA for 24 hr at 60°C. Filters were washed at room temperature in 2 x SSC and then at high stringency (0.1 x SSC, 30 min at 65°C) prior to autoradiography.

Example 11 Vectors for Local Synthesis of IGF IR-Regulating Oligonucleotides

Where up-regulation of IGF IR gene expression is desired, a fragment of the IGF IR cDNA clone of at least about 100 bp and containing the region surrounding the ATG start site is isolated and purified for insertion in the vector. These applications include stimulation of burn and other wound healing, angiogenesis and nerve regeneration.

Where down-regulation of IGF IR gene expression is desired, the XhoI-KpnI fragment of the IGF IR cDNA is used with insertion opposite in orientation to the expression control sequences (as in Example 3 herein). Other portions of the IGF IR cDNA of at least about 100 bp can be substituted. Such applications include prevention of postangioplasty restenosis or atherosclerosis of coronary arteries following cardiac transplant.

Control vectors are those lacking an IGF IR-specific insert. The vector used is a replication-deficient adenovirus vector (Stratford-Perricaudet et al. (1992) *J. Clin. Invest.* 90:626-630; Mastrangeli et al. (1993) *J. Clin. Invest.* 91:225-234) which is passaged in 293 cells (Mastrangeli et al. (1993) *supra*; Graham et al. (1973) *Virology* 52:456-467) to prepare virus stocks having $1-5 \times 10^{10}$ plaque-forming units/ml. Virus is administered hereinbelow.

Example 12 Trans-Catheter Delivery of Oligonucleotides

Domestic Yorkshire pigs (12-15 kg) are used as an animal model in tests of antisense oligonucleotide therapy to prevent postangioplasty restenosis. Pigs are anesthetized with zolazepam-tiletamine (6.0 mg/kg) in combination with intramuscular rompun (2.2 mg/kg) with 1% nitrous oxide.

The iliofemoral arteries are exposed using sterile surgical procedures, and a double-balloon catheter (C.R. Bard, Inc.) is inserted into the iliofemoral artery as previously described (Nabel et al. (1990) *Science* 249:1285; Nabel et al. (1989) *Science* 244:1342). The proximal balloon is inflated to 500 mmHg, as measured using an on-line pressure transducer, for 5 min. The balloon is then deflated and the catheter is advanced so that the central space between the proximal and distal balloons now

occupies the region of the previous injury. Both balloons are inflated, and the segment is irrigated with heparinized saline.

5 The antisense oligonucleotide preparations; antisense oligonucleotide (SEQ ID NO:1) or mismatch oligonucleotide control (SEQ ID NO:3) 1 mg per vessel, in saline, 2 ml volume, are instilled for 20 min in the central space in the catheter.

10 The catheter is removed, and antegrade blood flow is restored. The arteries are analyzed 21 or 42 days later to determine vascular proliferation and/or restenosis. After appropriate histological preparation, measurements of intimal (I) and medial (M) areas are determined in at least four sections from each artery, and the measurements from each artery are averaged. Comparisons of I/M area ratios for antisense-treated v. mismatch-treated are made using ANOVA with Dunnett's t test
15 (Dunnett, C.W. (1984) *Biometrics* 20:482). Statistical significance is assumed if a null hypothesis could be rejected at the 0.05 level.

20 Alternatively, in place of oligonucleotides, when inhibition of angiogenesis or vascular muscle proliferation is desired, a recombinant DNA molecule capable of directing the expression of IGF IR-specific antisense RNA and preferably only transiently expressed is administered by the above procedure. Such a recombinant molecule is described in Example 11 herein. The adenovirus vector described therein is replication-deficient and
25 therefore not stably maintained in the tissue. An adenovirus vector can be used at an exemplary dose of about 5×10^9 to 1×10^{10} plaque forming units, in a volume of about 0.7 ml.

Delivery of virus vectors is also described in Guzman et al. (1993) *Circulation* 88:2838-2848; Lemarchand et al. (1993) *Circulation Research* 72:1132-1138. Delivery of oligonucleotides
30 to the interior of blood vessels is described, e.g., in Shi et al. (1994) *Circulation* 90:944-951 and in Simon et al. (1992) *Nature* 359:67-70.

Example 13 Materials

35 Recombinant human IGF I was kindly provided by Dr. H.P. Guter, CIBA-GEIGY Corp., Summit, NJ [^3H]-thymidine (20 Ci/mmol),

[α -³²P]UTP (3000 Ci/mmol)], [α -³²P]dCTP (3000 Ci/mmol) and ¹²⁵I-IGF I (~300 μ Ci/ μ g), and [¹²⁵I-Sar¹-Ile⁸]-ang II (2200 Ci/mmol) were obtained from Du Pont-New England Nuclear (Boston, MA). Angiotensin II was purchased from Sigma Chemical Co. (St. Louis, MO).

Referring to SEQ ID NO:8, the cDNA sequence including the nucleotide sequence encoding rat IGF I receptor and SEQ ID NO:9, the deduced amino acid sequence, Applicant notes the following aspects: The coding sequence of the precursor protein extends from nucleotide 46 to nucleotide 4158, including the TGA stop codon within the encoded protein. The signal peptide is from amino acids -30 to -1 of SEQ ID NO:9. The α -subunit begins at amino acid 1. The C-terminus of the α -subunit is generated by protease cleavage at the 4-Arg proteolytic processing site (amino acids 708-711). The cysteine-rich region of the α -subunit is from amino acids 148 to 302. The β -subunit extends from amino acids 712 to 1340 of SEQ ID NO:9. The β -subunit transmembrane domain extends from amino acids 908 to 924, and the tyrosine kinase domain within the β -subunit extends from amino acids 976 to 1084 of SEQ ID NO:9.

With reference to SEQ ID NO:10 and SEQ ID NO:11, the precursor nucleotide sequence and deduced amino acid sequence for human IGF I receptor (from Ullrich et al. (1986) *EMBO Journal* 5:2503-2512) the coding sequence extends from nucleotide 46 to 4149 of SEQ ID NO:10 (including the stop codon). The signal peptide extends from amino acids -30 to -1 of SEQ ID NO:11. The mature α -subunit begins at amino acid 1 and extends to the proteolytic cleavage site at amino acids 707 to 710; the β -subunit begins at amino acid 711 and extends to amino acid 1337. The β -subunit transmembrane domain is at amino acids 906 to 929.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Delafontaine, Patrick
- 5 (ii) TITLE OF INVENTION: Methods for Regulation of Insulin-like
Growth Factor 1 Receptor
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
- 10 (A) ADDRESSEE: Greenlee and Winner, P.C.
(B) STREET: 5370 Manhattan Circle, Suite 201
(C) CITY: Boulder
(D) STATE: Colorado
(E) COUNTRY: U.S.A.
(F) ZIP: 80303
- 15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- 20 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO
(B) FILING DATE: 27-SEP-1995
(C) CLASSIFICATION:
- 25 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/317,898
(B) FILING DATE: 04-OCT-1994
- (viii) ATTORNEY/AGENT INFORMATION:
- 30 (A) NAME: Ferber, Donna M.
(B) REGISTRATION NUMBER: 33,878
(C) REFERENCE/DOCKET NUMBER: 48-94A PCT
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 499-8080
(B) TELEFAX: (303) 499-8089

(2) INFORMATION FOR SEQ ID NO:1:

- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCGGAGCCA GACTTCATTC

20

45 (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

35

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATGAAGTC TGGCTCCGGA

20

(2) INFORMATION FOR SEQ ID NO:3:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCGGTCCCA CTCTTGTTG

20

20 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30 CAGCTGCTGA TAGTCGTTGC

20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCAACGACTA TCAGCAGCTG

20

36

(2) INFORMATION FOR SEQ ID NO:6:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

10 CCCCAAATAA AAGGAATG

18

(2) INFORMATION FOR SEQ ID NO:7:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

20 AGCAGATTGC CCTTC

15

(2) INFORMATION FOR SEQ ID NO:8:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4696 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 30 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 46..4158
- (ix) FEATURE:
35 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 46..135
- (ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 136..4155

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

40 TTTTTTTTTT TTTGAGAAAA GGGAATTTTCG TCCCAAATAA AAGGA ATG AAG TCT 54
Met Lys Ser
-30

45 GGC TCC GGA GGA GGG TCC CCG ACC TCG CTG TGG GGG CTC GTG TTT CTC 102
Gly Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp ly Leu Val Phe Leu
-25 -20 -15

	TCC GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA ATC TGT GGG CCC	150
	Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile Cys Gly Pro	
	-10 -5 1 5	
5	GGC ATT GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAA AAC	198
	Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn	
	10 15 20	
	TGC ACG GTG ATC GAG GGC TTC CTC CAC ATC CTG CTC ATC TCC AAG GCC	246
	Cys Thr Val Ile Glu Gly Phe Leu His Ile Leu Leu Ile Ser Lys Ala	
	25 30 35	
10	GAG GAC TAC CGA AGC TAC CGC TTC CCC AAG CTC ACA GTC ATC ACC GAG	294
	Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu	
	40 45 50	
	TAC TTG CTG CTG TTT CGA GTG GCC GGC CTC GAG AGC CTG GAG GAC CTC	342
15	Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu	
	55 60 65	
	TTC CCG AAC CTC ACA GTC ATC CGT GGC TGG AAA CTC TTC TAC AAT TAC	390
	Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr	
	70 75 80 85	
20	GCA CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAT	438
	Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr	
	90 95 100	
	AAT CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAC GCT	486
	Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala	
	105 110 115	
25	GAC CTC TGT TAC CTC TCC ACC ATA GAC TGG TCT CTC ATC TTG GAT GCG	534
	Asp Leu Cys Tyr Leu Ser Thr Ile Asp Trp Ser Leu Ile Leu Asp Ala	
	120 125 130	
	GTG TCC AAT AAC TAC ATT GTG GGG AAC AAG CCC CCA AAG GAA TGT GGG	582
30	Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly	
	135 140 145	
	GAC CTG TGT CCA GGG ACC TTG GAG GAG AAG CCC ATG TGT GAG AAG ACC	630
	Asp Leu Cys Pro Gly Thr Leu Glu Glu Lys Pro Met Cys Glu Lys Thr	
	150 155 160 165	
35	ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAT CGC TGC	678
	Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys	
	170 175 180	
	CAG AAA ATG TGC CCA AGT GTG TGT GGG AAG CGA GCC TGC ACC GAG AAC	726
	Gln Lys Met Cys Pro Ser Val Cys Gly Lys Arg Ala Cys Thr Glu Asn	
	185 190 195	
40	AAT GAG TGC TGC CAC CCG GAG TGC CTA GGC AGC TGC CAC ACA CCG GAC	774
	Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys His Thr Pro Asp	
	200 205 210	
	GAC AAC ACA ACC TGC GTG GCC TGC CGA CAC TAC TAC TAC AAA GGC GTG	822
45	Asp Asn Thr Thr Cys Val Ala Cys Arg His Tyr Tyr Tyr Lys Gly Val	
	215 220 225	
	TGC GTG CCT GCC TGC CCG CCT GCG ACC TAC AGG TTC GAG GGC TGG CGC	870
	Cys Val Pro Ala Cys Pro Pro Gly Thr Tyr Arg Phe Glu Gly Trp Arg	
	230 235 240 245	
50	TGT GTG GAC CGG GAT TTC TGC GCC AAC ATC CCC AAC GCC GAG AGC AGT	918
	Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Pro Asn Ala Glu Ser Ser	
	250 255 260	

38

		GAC	TCA	GAT	GGC	TTC	GTC	ATC	CAC	GAT	GGC	GAG	TGC	ATG	CAG	GAG	TGT	966
		Asp	Ser	Asp	Gly	Phe	Val	Ile	His	Asp	Gly	Glu	Cys	Met	Gln	Glu	Cys	
					265					270					275			
5		CCA	TCA	GGC	TTC	ATC	CGC	AAC	AGC	ACC	CAG	AGC	ATG	TAC	TGT	ATC	CCC	1014
		Pro	Ser	Gly	Phe	Ile	Arg	Asn	Ser	Thr	Gln	Ser	Met	Tyr	Cys	Ile	Pro	
				280				285						290				
		TGT	GAA	GGC	CCC	TGC	CCC	AAG	GTC	TGC	GGC	GAT	GAA	GAA	AAG	AAA	ACG	1062
		Cys	Glu	Gly	Pro	Cys	Pro	Lys	Val	Cys	Gly	Asp	Glu	Glu	Lys	Lys	Thr	
			295					300					305					
10		AAA	ACC	ATC	GAT	TCT	GTG	ACG	TCT	GCC	CAG	ATG	CTC	CAA	GGG	TGC	ACC	1110
		Lys	Thr	Ile	Asp	Ser	Val	Thr	Ser	Ala	Gln	Met	Leu	Gln	Gly	Cys	Thr	
		310					315					320					325	
		ATT	TTG	AAG	GGC	AAT	CTG	CTT	ATT	AAC	ATC	CGG	CGA	GGC	AAT	AAC	ATT	1158
15		Ile	Leu	Lys	Gly	Asn	Leu	Leu	Ile	Asn	Ile	Arg	Arg	Gly	Asn	Asn	Ile	
						330					335					340		
		GCC	TCG	GAA	TTG	GAG	AAC	TTC	ATG	GGG	CTC	ATC	GAG	GTG	GTG	ACT	GGC	1206
		Ala	Ser	Glu	Leu	Glu	Asn	Phe	Met	Gly	Leu	Ile	Glu	Val	Val	Thr	Gly	
					345					350					355			
20		TAC	GTG	AAG	ATC	CGC	CAT	TCC	CAT	GCC	TTG	GTC	TCC	TTG	TCC	TTC	CTG	1254
		Tyr	Val	Lys	Ile	Arg	His	Ser	His	Ala	Leu	Val	Ser	Leu	Ser	Phe	Leu	
				360				365						370				
		AAG	AAC	CTT	CGT	CTC	ATC	TTA	GGA	GAG	GAG	CAG	CTA	GAA	GGA	AAC	TAC	1302
		Lys	Asn	Leu	Arg	Leu	Ile	Leu	Gly	Glu	Glu	Gln	Leu	Glu	Gly	Asn	Tyr	
			375					380					385					
25		TCC	TTC	TAT	GTC	CTG	GAC	AAC	CAG	AAC	TTG	CAG	CAG	CTG	TGG	GAC	TGG	1350
		Ser	Phe	Tyr	Val	Leu	Asp	Asn	Gln	Asn	Leu	Gln	Gln	Leu	Trp	Asp	Trp	
		390					395					400					405	
		AAC	CAC	CGG	AAC	CTG	ACC	GTC	AGG	TCA	GGG	AAA	ATG	TAC	TTC	GCT	TTC	1398
30		Asn	His	Arg	Asn	Leu	Thr	Val	Arg	Ser	Gly	Lys	Met	Tyr	Phe	Ala	Phe	
					410						415					420		
		AAT	CCC	AAG	CTG	TGT	GTC	TCT	GAA	ATT	TAC	CGA	ATG	GAG	GAG	GTG	ACA	1446
		Asn	Pro	Lys	Leu	Cys	Val	Ser	Glu	Ile	Tyr	Arg	Met	Glu	Glu	Val	Thr	
				425					430					435				
35		GGA	ACA	AAG	GGA	CGG	CAG	AGC	AAA	GGA	GAC	ATA	AAC	ACC	AGG	AAC	AAC	1494
		Gly	Thr	Lys	Gly	Arg	Gln	Ser	Lys	Gly	Asp	Ile	Asn	Thr	Arg	Asn	Asn	
				440				445						450				
		GGA	GAG	CGA	GCT	TCC	TGT	GAA	AGT	GAT	GTT	CTC	CGT	TTC	ACC	TCC	ACC	1542
		Gly	Glu	Arg	Ala	Ser	Cys	Glu	Ser	Asp	Val	Leu	Arg	Phe	Thr	Ser	Thr	
			455					460					465					
40		ACC	ACC	TGG	AAG	AAC	CGC	ATC	ATC	ATA	ACG	TGG	CAC	CGG	TAC	CGG	CCG	1590
		Thr	Thr	Trp	Lys	Asn	Arg	Ile	Ile	Ile	Thr	Trp	His	Arg	Tyr	Arg	Pro	
		470					475					480					485	
		CCG	GAC	TAC	CGG	GAT	CTC	ATC	AGT	TTC	ACA	GTC	TAC	TAC	AAG	GAG	GCA	1638
45		Pro	Asp	Tyr	Arg	Asp	Leu	Ile	Ser	Phe	Thr	Val	Tyr	Tyr	Lys	Glu	Ala	
					490						495					500		
		CCC	TTT	AAA	AAC	GTC	ACG	GAA	TAC	GAC	GGG	CAG	GAT	GCC	TGT	GGC	TCC	1686
		Pro	Phe	Lys	Asn	Val	Thr	Ile	Tyr	Asp	Gly	Gln	Asp	Ala	Cys	Gly	Ser	
				505						510					515			
50		AAC	AGC	TGG	AAC	ATG	GTG	GAC	GTG	GAC	CTG	CCT	CCG	AAC	AAG	GAG	GGG	1734
		Asn	Ser	Trp	Asn	Met	Val	Asp	Val	Asp	Leu	Pro	Pro	Asn	Lys	Glu	Gly	
				520				525						530				

	GAG CCT GGC ATT TTG CTG CAT GGG CTG AAG CCC TGG ACC CAG TAT GCA	1782
	Glu Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln Tyr Ala	
	535 540 545	
5	GTC TAT GTC AAG GCT GTG ACC CTC ACC ATG GTG GAA AAC GAC CAC ATC	1830
	Val Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile	
	550 555 560 565	
	CGT GGG GCC AAA AGT GAA ATC TTG TAC ATT CGC ACC AAC GCT TCA GTT	1878
	Arg Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val	
	570 575 580	
10	CCT TCC ATT CCT CTA GAT GTC CTC TCG GCA TCA AAC TCC TCC TCT CAG	1926
	Pro Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln	
	585 590 595	
	CTG ATC GTG AAG TGG AAC CCC CCA ACT CTG CCC AAT GGT AAC TTG AGT	1974
15	Leu Ile Val Lys Trp Asn Pro Pro Thr Leu Pro Asn Gly Asn Leu Ser	
	600 605 610	
	TAC TAC ATT GTG AGG TGG CAG CGG CAG CCG CAG GAT GGC TAT CTG TTC	2022
	Tyr Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Phe	
	615 620 625	
20	CGG CAC AAC TAC TGC TCC AAA GAC AAA ATA CCC ATC AGA AAG TAC GCC	2070
	Arg His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala	
	630 635 640 645	
	GAT GGT ACC ATC GAT GTG GAG GAG GTG ACA GAA AAT CCC AAG ACA GAA	2118
	Asp Gly Thr Ile Asp Val Glu Glu Val Thr Glu Asn Pro Lys Thr Glu	
	650 655 660	
25	GTC TGC GGT GGT GAT AAA GGG CCG TGC TGT GCC TGT CCT AAA ACC GAA	2166
	Val Cys Gly Gly Asp Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu	
	665 670 675	
	GCT GAG AAG CAG GCT GAG AAG GAG GAG GCT GAG TAC CGT AAA GTC TTT	2214
30	Ala Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe	
	680 685 690	
	GAG AAT TTC CTT CAC AAC TCC ATC TTT GTG CCC AGA CCT GAG AGG AGG	2262
	Glu Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Arg	
	695 700 705	
35	CGG AGA GAT GTC CTG CAG GTG GCT AAC ACC ACC ATG TCC AGC CGA AGC	2310
	Arg Arg Asp Val Leu Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser	
	710 715 720 725	
	AGG AAC ACC ACG GTA GCT GAC ACC TAC AAT ATC ACA GAC CCG GAA GAG	2358
	Arg Asn Thr Thr Val Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu	
	730 735 740	
40	TTC GAG ACA GAA TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC AAG GAG	2406
	Phe Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Lys Glu	
	745 750 755	
	AGG ACT GTC ATT TCC AAC CTC CGG CCT TTC ACT CTG TAC CGT ATC GAT	2454
45	Arg Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Arg Ile Asp	
	760 765 770	
	ATC CAC AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC	2502
	Ile His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser	
	775 780 785	
50	AAC TTT GTC TTT GCA AGA ACC ATG CCA GCA GAA GGA GCA GAT GAC ATT	2550
	Asn Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile	
	790 795 800 805	

40

	CCT GGC CCA GTG ACC TGG GAG CCA AGA CCT GAA AAC TCC ATC TTT TTA	2598
	Pro Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu	
	810 815 820	
5	AAG TGG CCA GAA CCC GAG AAC CCC AAC GGA TTG ATT CTA ATG TAT GAA	2646
	Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met Tyr Glu	
	825 830 835	
	ATA AAA TAC GGA TCG CAA GTC GAG GAT CAG CGG GAA TGT GTG TCC AGA	2694
	Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val Ser Arg	
	840 845 850	
10	CAG GAG TAC AGG AAG TAT GGA GGG GCC AAA CTT AAC CGT CTA AAC CCA	2742
	Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu Asn Pro	
	855 860 865	
15	GGG AAC TAT ACG GCC CGG ATT CAG GCT ACC TCC CTC TCT GGG AAT GGG	2790
	Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly Asn Gly	
	870 875 880 885	
	TCG TGG ACA GAT CCT GTG TTC TTC TAT GTC CCA GCC AAA ACA ACG TAT	2838
	Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Pro Ala Lys Thr Thr Tyr	
	890 895 900	
20	GAG AAT TTC ATG CAT CTG ATC ATT GCT CTG CCG GTT GCC ATC CTG CTG	2886
	Glu Asn Phe Met His Leu Ile Ile Ala Leu Pro Val Ala Ile Leu Leu	
	905 910 915	
	ATT GTG GGG GGC CTG GTA ATC ATG CTG TAT GTC TTC CAT AGA AAG AGG	2934
	Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg Lys Arg	
	920 925 930	
25	AAT AAC AGC AGA TTG GGC AAC GGG GTG CTG TAC GCC TCT GTG AAC CCC	2982
	Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val Asn Pro	
	935 940 945	
30	GAG TAT TTC AGC GCA GCT GAT GTG TAC GTG CCT GAT GAA TGG GAG GTA	3030
	Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp Glu Val	
	950 955 960 965	
	GCT CGG GAG AAG ATC ACC ATG AAC CCG GAG CTC GGA CAA GGG TCC TTC	3078
	Ala Arg Glu Lys Ile Thr Met Asn Arg Glu Leu Gly Gln Gly Ser Phe	
	970 975 980	
35	GGG ATG GTC TAT GAA GGA GTG GCC AAG GGC GTG GTC AAG GAC GAG CCT	3126
	Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp Glu Pro	
	985 990 995	
	GAA ACC AGA GTG GCC ATC AAG ACA GTG AAT GAG GCT GCA AGT ATG CGT	3174
	Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser Met Arg	
	1000 1005 1010	
40	GAG AGA ATT GAG TTT CTC AAC GAG GCC TCA GTG ATG AAG GAG TTC AAC	3222
	Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn	
	1015 1020 1025	
	TGT CAC CAT GTG GTC CGG TTG CTG GGT GTA GTA TCC CAA GGC CAG CCC	3270
	Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly Gln Pro	
45	1030 1035 1040 1045	
	ACC CTG GTC ATC ATG GAA CTA ATG ACA CGT GGC GAT CTC AAA AGT TAT	3318
	Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys Ser Tyr	
	1050 1055 1060	
50	CTC CGG TCT CTA AGG CCA GAG GTG GAG AAT AAT CTA GTC CTG ATT CCT	3366
	Leu Arg Ser Leu Arg Pro Glu Val Glu Asn Asn Leu Val Leu Ile Pro	
	1065 1070 1075	

	CCG AGC TTA AGC AAG ATG ATC CAG ATG GCT GGA GAG ATT GCA GAT GGC Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala Asp Gly 1080 1085 1090	3414
5	ATG GCC TAC CTC AAT GCC AAC AAG TTC GTC CAC AGA GAC CTG GCT GCT Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala 1095 1100 1105	3462
	CGG AAC TGC ATG GTA GCT GAA GAT TTC ACA GTC AAA ATT GGA GAT TTT Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe 1110 1115 1120 1125	3510
10	GGT ATG ACA CGA GAC ATC TAC GAG ACG GAC TAC TAC CGG AAA GGC GGC Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly 1130 1135 1140	3558
15	AAG GGC TTG CTG CCT GTG CGC TGG ATG TCT CCC GAG TCC CTC AAG GAT Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp 1145 1150 1155	3606
	GGC GTC TTC ACC ACT CAT TCC GAT GTC TGG TCC TTT GGG GTC GTC CTC Gly Val Phe Thr Thr His Ser Asp Val Trp Ser Phe Gly Val Val Leu 1160 1165 1170	3654
20	TGG GAG ATC GCC ACT CTG GCT GAG CAG CCG TAC CAG GGC CTG TCC AAC Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn 1175 1180 1185	3702
	GAG CAA GTT CTT CGT TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCG Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro 1190 1195 1200 1205	3750
25	GAT AAC TGC CCC GAT ATG CTG TTT GAA CTT ATG CGC ATG TGC TGG CAG Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln 1210 1215 1220	3798
30	TAC AAC CCC AAG ATG CGG CCC TCC TTC CTG GAG ATC ATC GGA AGC ATC Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile GGA AGC ATC 1225 1230 1235	3846
	AAG GAT GAG ATG GAG CCC AGT TTC CAG GAG GTC TCC TTC TAC TAC AGC Lys Asp Glu Met Glu Pro Ser Phe Gln Glu Val Ser Phe Tyr Tyr Ser 1240 1245 1250	3894
35	GAG GAG AAC AAG CCT CCA GAG CCG GAG GAG CTG GAG ATG GAG CTG GAG Glu Glu Asn Lys Pro Pro Glu Pro Glu Glu Leu Glu Met Glu Leu Glu 1255 1260 1265	3942
	CTG GAG CCC GAG AAC ATG GAG AGC GTC CCG CTG GAC CCT TCG GCC TCC Leu Glu Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser 1270 1275 1280 1285	3990
40	TCA GCC TCC CTG CCT CTG CCT GAA AGA CAC TCA GGA CAC AAG GCT GAG Ser Ala Ser Leu Pro Leu Pro Glu Arg His Ser Gly His Lys Ala Glu 1290 1295 1300	4038
45	AAC GGC CCT GGC GTG CTG GTT CTC CGT GCC AGT TTT GAT GAG AGA CAG Asn Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln 1305 1310 1315	4086
	CCT TAC GCT CAC ATG AAT GGG GGA CGC GCC AAC GAG AGG GCC TTG CCT Pro Tyr Ala His Met Asn Gly Gly Arg Ala Asn Glu Arg Ala Leu Pro 1320 1325 1330	4134
50	CTG CCC CAG TCC TCG ACC TGC TGA TCCTCGACCC CGAAGCACGT GCAAACAGTA Leu Pro Gln Ser Ser Thr Cys * 1335 1340	4188

	ACGTGTGTGT GTGTGTGCGT GCGTGCGTGT GTGCTCACTC GGTGGGCGGA GGGGGGAGCA	4248
	GGTTGTAACA ATCTATTCAC AAGCCTCCTG TATCTCAGTG GATCTTCAGA ACTGCCCTTG	4308
	CTGCCCACGG GAGTCGGCTT CTCTGCAGTA AACACACTTG GGACCTTCCT TTTTTCATA	4368
	TGCAAGCAGC TTTTTTTTATT TCCCTACCCA AACCCCTTAAC TGACATGGGC CTCTGCAAAC	4428
5	CTTAATGACA GCTCTTAATA GCAACAGGAC ACTCGAGAAT TGAGTCTCCT CATTCTCTGC	4488
	CTTTTCTCTC TTCTGCCTTC CCTCTCTCTC CTCTCCCTTC CCACTTCCAG GCTCTCCTCT	4548
	CTTTTCTCCG CTTCCTCAGTG GAAAGCCCAG CGGGGAAGCC TTTATTTTATT TTTTITTTAA	4608
	ATCAAATTGA CTTAATGGCT GCCCTGGGGC TCCTCATCAC ACCTGCCTGA GCACCATAGG	4668
	TCTTTACAAA AAAGAAAGGA AAAAAACC	4696
10	(2) INFORMATION FOR SEQ ID NO:9:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 1371 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
15	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
	Met Lys Ser Gly Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp Gly Leu	
	-30 -25 -20 -15	
20	Val Phe Leu Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile	
	-10 -5 1	
	Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg	
	5 10 15	
	Leu Glu Asn Cys Thr Val Ile Glu Gly Phe Leu His Ile Leu Leu Ile	
	20 25 30	
25	Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val	
	35 40 45 50	
	Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu	
	55 60 65	
30	Gly Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe	
	70 75 80	
	Tyr Asn Tyr Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile	
	85 90 95	
	Gly Leu Tyr Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu	
	100 105 110	
35	Lys Asn Ala Asp Leu Cys Tyr Leu Ser Thr Ile Asp Trp Ser Leu Ile	
	115 120 125 130	
	Leu Asp Ala Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys	
	135 140 145	
40	Glu Cys Gly Asp Leu Cys Pro Gly Thr Leu Glu Glu Lys Pro Met Cys	
	150 155 160	

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	Glu	Lys	Thr	Thr	Ile	Asn	Asn	Glu	Tyr	Asn	Tyr	Arg	Cys	Trp	Thr	Thr	
			165					170					175				
	Asn	Arg	Cys	Gln	Lys	Met	Cys	Pro	Ser	Val	Cys	Gly	Lys	Arg	Ala	Cys	
	180						185					190					
5	Thr	Glu	Asn	Asn	Glu	Cys	Cys	His	Pro	Glu	Cys	Leu	Gly	Ser	Cys	His	
	195					200					205					210	
	Thr	Pro	Asp	Asp	Asn	Thr	Thr	Cys	Val	Ala	Cys	Arg	His	Tyr	Tyr	Tyr	
					215					220					225		
10	Lys	Gly	Val	Cys	Val	Pro	Ala	Cys	Pro	Pro	Gly	Thr	Tyr	Arg	Phe	Glu	
				230					235					240			
	Gly	Trp	Arg	Cys	Val	Asp	Arg	Asp	Phe	Cys	Ala	Asn	Ile	Pro	Asn	Ala	
			245					250					255				
	Glu	Ser	Ser	Asp	Ser	Asp	Gly	Phe	Val	Ile	His	Asp	Gly	Glu	Cys	Met	
	260						265					270					
15	Gln	Glu	Cys	Pro	Ser	Gly	Phe	Ile	Arg	Asn	Ser	Thr	Gln	Ser	Met	Tyr	
	275					280					285					290	
	Cys	Ile	Pro	Cys	Glu	Gly	Pro	Cys	Pro	Lys	Val	Cys	Gly	Asp	Glu	Glu	
					295					300					305		
20	Lys	Lys	Thr	Lys	Thr	Ile	Asp	Ser	Val	Thr	Ser	Ala	Gln	Met	Leu	Gln	
				310					315					320			
	Gly	Cys	Thr	Ile	Leu	Lys	Gly	Asn	Leu	Leu	Ile	Asn	Ile	Arg	Arg	Gly	
			325					330					335				
	Asn	Asn	Ile	Ala	Ser	Glu	Leu	Glu	Asn	Phe	Met	Gly	Leu	Ile	Glu	Val	
	340						345					350					
25	Val	Thr	Gly	Tyr	Val	Lys	Ile	Arg	His	Ser	His	Ala	Leu	Val	Ser	Leu	
	355					360					365					370	
	Ser	Phe	Leu	Lys	Asn	Leu	Arg	Leu	Ile	Leu	Gly	Glu	Glu	Gln	Leu	Glu	
				375						380					385		
30	Gly	Asn	Tyr	Ser	Phe	Tyr	Val	Leu	Asp	Asn	Gln	Asn	Leu	Gln	Gln	Leu	
				390					395					400			
	Trp	Asp	Trp	Asn	His	Arg	Asn	Leu	Thr	Val	Arg	Ser	Gly	Lys	Met	Tyr	
			405					410					415				
	Phe	Ala	Phe	Asn	Pro	Lys	Leu	Cys	Val	Ser	Glu	Ile	Tyr	Arg	Met	Glu	
	420						425					430					
35	Glu	Val	Thr	Gly	Thr	Lys	Gly	Arg	Gln	Ser	Lys	Gly	Asp	Ile	Asn	Thr	
	435					440					445					450	
	Arg	Asn	Asn	Gly	Glu	Arg	Ala	Ser	Cys	Glu	Ser	Asp	Val	Leu	Arg	Phe	
				455						460					465		
40	Thr	Ser	Thr	Thr	Thr	Trp	Lys	Asn	Arg	Ile	Ile	Ile	Thr	Trp	His	Arg	
				470					475					480			
	Tyr	Arg	Pro	Pro	Asp	Tyr	Arg	Asp	Leu	Ile	Ser	Phe	Thr	Val	Tyr	Tyr	
			485					490					495				
	Lys	Glu	Ala	Pro	Phe	Lys	Asn	Val	Thr	Glu	Tyr	Asp	Gly	Gln	Asp	Ala	
	500						505					510					

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	Cys	Gly	Ser	Asn	Ser	Trp	Asn	Met	Val	Asp	Val	Asp	Leu	Pro	Pro	Asn
	515					520					525					530
	Lys	Glu	Gly	Glu	Pro	Gly	Ile	Leu	Leu	His	Gly	Leu	Lys	Pro	Trp	Thr
					535					540					545	
5	Gln	Tyr	Ala	Val	Tyr	Val	Lys	Ala	Val	Thr	Leu	Thr	Met	Val	Glu	Asn
				550					555					560		
	Asp	His	Ile	Arg	Gly	Ala	Lys	Ser	Glu	Ile	Leu	Tyr	Ile	Arg	Thr	Asn
			565					570					575			
10	Ala	Ser	Val	Pro	Ser	Ile	Pro	Leu	Asp	Val	Leu	Ser	Ala	Ser	Asn	Ser
	580						585					590				
	Ser	Ser	Gln	Leu	Ile	Val	Lys	Trp	Asn	Pro	Pro	Thr	Leu	Pro	Asn	Gly
	595					600					605					610
	Asn	Leu	Ser	Tyr	Tyr	Ile	Val	Arg	Trp	Gln	Arg	Gln	Pro	Gln	Asp	Gly
					615					620					625	
15	Tyr	Leu	Phe	Arg	His	Asn	Tyr	Cys	Ser	Lys	Asp	Lys	Ile	Pro	Ile	Arg
				630					635					640		
	Lys	Tyr	Ala	Asp	Gly	Thr	Ile	Asp	Val	Glu	Glu	Val	Thr	Glu	Asn	Pro
			645					650					655			
20	Lys	Thr	Glu	Val	Cys	Gly	Gly	Asp	Lys	Gly	Pro	Cys	Cys	Ala	Cys	Pro
	660						665					670				
	Lys	Thr	Glu	Ala	Glu	Lys	Gln	Ala	Glu	Lys	Glu	Glu	Ala	Glu	Tyr	Arg
	675					680					685					690
	Lys	Val	Phe	Glu	Asn	Phe	Leu	His	Asn	Ser	Ile	Phe	Val	Pro	Arg	Pro
					695					700					705	
25	Glu	Arg	Arg	Arg	Arg	Asp	Val	Leu	Gln	Val	Ala	Asn	Thr	Thr	Met	Ser
				710					715						720	
	Ser	Arg	Ser	Arg	Asn	Thr	Thr	Val	Ala	Asp	Thr	Tyr	Asn	Ile	Thr	Asp
			725					730					735			
30	Pro	Glu	Glu	Phe	Glu	Thr	Glu	Tyr	Pro	Phe	Phe	Glu	Ser	Arg	Val	Asp
	740						745					750				
	Asn	Lys	Glu	Arg	Thr	Val	Ile	Ser	Asn	Leu	Arg	Pro	Phe	Thr	Leu	Tyr
	755					760					765					770
	Arg	Ile	Asp	Ile	His	Ser	Cys	Asn	His	Glu	Ala	Glu	Lys	Leu	Gly	Cys
					775					780					785	
35	Ser	Ala	Ser	Asn	Phe	Val	Phe	Ala	Arg	Thr	Met	Pro	Ala	Glu	Gly	Ala
				790					795					800		
	Asp	Asp	Ile	Pro	Gly	Pro	Val	Thr	Trp	Glu	Pro	Arg	Pro	Glu	Asn	Ser
			805					810					815			
40	Ile	Phe	Leu	Lys	Trp	Pro	Glu	Pro	Glu	Asn	Pro	Asn	Gly	Leu	Ile	Leu
	820						825					830				
	Met	Tyr	Glu	Ile	Lys	Tyr	Gly	Ser	Gln	Val	Glu	Asp	Gln	Arg	Glu	Cys
	835					840					845					850
	Val	Ser	Arg	Gln	Glu	Tyr	Arg	Lys	Tyr	Gly	Gly	Ala	Lys	Leu	Asn	Arg
					855					860					865	

45

Leu Asn Pro Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser
 870 875 880

Gly Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Pro Ala Lys
 885 890 895

5 Thr Thr Tyr Glu Asn Phe Met His Leu Ile Ile Ala Leu Pro Val Ala
 900 905 910

Ile Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His
 915 920 925 930

10 Arg Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser
 935 940 945

Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu
 950 955 960

Trp Glu Val Ala Arg Glu Lys Ile Thr Met Asn Arg Glu Leu Gly Gln
 965 970 975

15 Gly Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys
 980 985 990

Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala
 995 1000 1005 1010

20 Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys
 1015 1020 1025

Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln
 1030 1035 1040

Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu
 1045 1050 1055

25 Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Val Glu Asn Asn Leu Val
 1060 1065 1070

Leu Ile Pro Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile
 1075 1080 1085 1090

30 Ala Asp Gly Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp
 1095 1100 1105

Leu Ala Ala Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile
 1110 1115 1120

Gly Asp Phe Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg
 1125 1130 1135

35 Lys Gly Gly Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser
 1140 1145 1150

Leu Lys Asp Gly Val Phe Thr Thr His Ser Asp Val Trp Ser Phe Gly
 1155 1160 1165 1170

40 Val Val Leu Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly
 1175 1180 1185

Leu Ser Asn Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu
 1190 1195 1200

Asp Lys Pro Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met
 1205 1210 1215

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Cys Trp Gln Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile
 1220 1225 1230
 Gly Ser Ile Lys Asp Glu Met Glu Pro Ser Phe Gln Glu Val Ser Phe
 1235 1240 1245 1250
 5 Tyr Tyr Ser Glu Glu Asn Lys Pro Pro Glu Pro Glu Glu Leu Glu Met
 1255 1260 1265
 Glu Leu Glu Leu Glu Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro
 1270 1275 1280
 10 Ser Ala Ser Ser Ala Ser Leu Pro Leu Pro Glu Arg His Ser Gly His
 1285 1290 1295
 Lys Ala Glu Asn Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp
 1300 1305 1310
 Glu Arg Gln Pro Tyr Ala His Met Asn Gly Gly Arg Ala Asn Glu Arg
 1315 1320 1325 1330
 15 Ala Leu Pro Leu Pro Gln Ser Ser Thr Cys *
 1335 1340

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 20 (A) LENGTH: 4989 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 46..4149

30 (ix) FEATURE:

(A) NAME/KEY: sig_peptide
 (B) LOCATION: 46..135

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 136..4146

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTTTTTTTT TTTTGAGAAA GCGAATTTC A TCCCAAATAA AAGGA ATG AAG TCT 54
 Met Lys Ser
 -30
 40 GGC TCC GGA GGA GGG TCC CCG ACC TCG GTG TGG GGG CTC CTG TTT CTC 102
 Gly Ser Gly Gly Ser Pro Thr Ser Val Trp Gly Leu Leu Phe Leu
 -25 -20 -15
 TCC GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA ATC TGC GGG CCA 150
 Ser Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile Cys Gly Pro
 -10 -5 1 5

47

	GGC ATC GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAG AAC Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn 10 15 20	198
5	TGC ACG GTG ATC GAG GGC TAC CTC CAC ATC CTG CTC ATC TCC AAG GCC Cys Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile Ser Lys Ala 25 30 35	246
	GAG GAC TAC CGC AGC TAC CGC TTC CCC AAG CTC ACG GTC ATT ACC GAG Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu 40 45 50	294
10	TAC TTG CTG CTG TTC CGA GTG GCT GGC CTC GAG AGC CTC GGA GAC CTC Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu 55 60 65	342
15	TTC CCC AAC CTC ACG GTC ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr 70 75 80 85	390
	GCC CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr 90 95 100	438
20	AAC CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala 105 110 115	486
	GAC CTC TGT TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG Asp Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala 120 125 130	534
25	GTG TCC AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly 135 140 145	582
30	GAC CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC Asp Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr 150 155 160 165	630
	ACC ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC TGC Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys 170 175 180	678
35	CAG AAA ATG TGC CCA AGC ACG TGT GGG AAG CCG GCG TGC ACC GAG AAC Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr Glu Asn 185 190 195	726
	AAT GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC GCG CCT GAC Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser Ala Pro Asp 200 205 210	774
40	AAC GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC TAT GCC GGT GTC Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr Ala Gly Val 215 220 225	822
45	TGT GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG TTT GAG GGC TGG CGC Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu Gly Trp Arg 230 235 240 245	870
	TGT GTG GAC CGT GAC TTC TGC GCC AAC ATC CTC AGC GCC GAG AGC AGC Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala Glu Ser Ser 250 255 260	918
50	GAC TCC GAG GGG TTT GTG ATC CAC GAC GGC GAG TGC TGC CAG GAG TGC Asp Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met Gln Glu Cys 265 270 275	966

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	CCC	TCG	GGC	TTC	ATC	CGC	AAC	GGC	AGC	CAG	AGC	ATG	TAC	TGC	ATC	CCT	1014
	Pro	Ser	Gly	Phe	Ile	Arg	Asn	Gly	Ser	Gln	Ser	Met	Tyr	Cys	Ile	Pro	
			280					285					290				
5	TGT	GAA	GGT	CCT	TGC	CCG	AAG	GTC	TGT	GAG	GAA	GAA	AAG	AAA	ACA	AAG	1062
	Cys	Glu	Gly	Pro	Cys	Pro	Lys	Val	Cys	Glu	Glu	Glu	Lys	Lys	Thr	Lys	
		295					300				305						
	ACC	ATT	GAT	TCT	GTT	ACT	TCT	GCT	CAG	ATG	CTC	CAA	GGA	TGC	ACC	ATC	1110
	Thr	Ile	Asp	Ser	Val	Thr	Ser	Ala	Gln	Met	Leu	Gln	Gly	Cys	Thr	Ile	
	310					315					320					325	
10	TTC	AAG	GGC	AAT	TTG	CTC	ATT	AAC	ATC	CGA	CGG	GGG	AAT	AAC	ATT	GCT	1158
	Phe	Lys	Gly	Asn	Leu	Ile	Asn	Ile	Arg	Arg	Gly	Asn	Asn	Ile	Ala		
				330					335						340		
	TCA	GAG	CTG	GAG	AAC	TTC	ATG	GGG	CTC	ATC	GAG	GTG	GTG	ACG	GGC	TAC	1206
15	Ser	Glu	Leu	Glu	Asn	Phe	Met	Gly	Leu	Ile	Glu	Val	Val	Thr	Gly	Tyr	
			345					350						355			
	GTG	AAG	ATC	CGC	CAT	TCT	CAT	GCC	TTG	GTC	TCC	TTG	TCC	TTC	CTA	AAA	1254
	Val	Lys	Ile	Arg	His	Ser	His	Ala	Leu	Val	Ser	Leu	Ser	Phe	Leu	Lys	
			360					365					370				
20	AAC	CTT	CGC	CTC	ATC	CTA	GGA	GAG	GAG	CAG	CTA	GAA	GGG	AAT	TAC	TCC	1302
	Asn	Leu	Arg	Leu	Ile	Leu	Gly	Glu	Glu	Gln	Leu	Glu	Gly	Asn	Tyr	Ser	
		375				380						385					
	TTC	TAC	GTC	CTC	GAC	AAC	CAG	AAC	TTG	CAG	CAA	CTG	TGG	GAC	TGG	GAC	1350
	Phe	Tyr	Val	Leu	Asp	Asn	Gln	Asn	Leu	Gln	Gln	Leu	Trp	Asp	Trp	Asp	
	390				395					400						405	
25	CAC	CGC	AAC	CTG	ACC	ATC	AAA	GCA	GGG	AAA	ATG	TAC	TTT	GCT	TTC	AAT	1398
	His	Arg	Asn	Leu	Thr	Ile	Lys	Ala	Gly	Lys	Met	Tyr	Phe	Ala	Phe	Asn	
				410					415						420		
	CCC	AAA	TTA	TGT	GTT	TCC	GAA	ATT	TAC	CGC	ATG	GAG	GAA	GTG	ACG	GGG	1446
30	Pro	Lys	Leu	Cys	Val	Ser	Glu	Ile	Tyr	Arg	Met	Glu	Glu	Val	Thr	Gly	
			425						430					435			
	ACT	AAA	GGG	CGC	CAA	AGC	AAA	GGG	GAC	ATA	AAC	ACC	AGG	AAC	AAC	GGG	1494
	Thr	Lys	Gly	Arg	Gln	Ser	Lys	Gly	Asp	Ile	Asn	Thr	Arg	Asn	Asn	Gly	
			440					445					450				
35	GAG	AGA	GCC	TCC	TGT	GAA	AGT	GAC	GTC	CTG	CAT	TTC	ACC	TCC	ACC	ACC	1542
	Glu	Arg	Ala	Ser	Cys	Glu	Ser	Asp	Val	Leu	His	Phe	Thr	Ser	Thr	Thr	
		455				460						465					
	ACG	TCG	AAG	AAT	CGC	ATC	ATC	ATA	ACC	TGG	CAC	CGG	TAC	CGG	CCC	CCT	1590
	Thr	Ser	Lys	Asn	Arg	Ile	Ile	Ile	Thr	Trp	His	Arg	Tyr	Arg	Pro	Pro	
	470				475						480					485	
40	GAC	TAC	AGG	GAT	CTC	ATC	AGC	TTC	ACC	GTT	TAC	TAC	AAG	GAA	GCA	CCC	1638
	Asp	Tyr	Arg	Asp	Leu	Ile	Ser	Phe	Thr	Val	Tyr	Tyr	Lys	Glu	Ala	Pro	
				490						495					500		
	TTT	AAG	AAT	GTC	ACA	GAG	TAT	GAT	GGG	CAG	GAT	GCC	TGC	GGC	TCC	AAC	1686
45	Phe	Lys	Asn	Val	Thr	Glu	Tyr	Asp	Gly	Gln	Asp	Ala	Cys	Gly	Ser	Asn	
			505					510						515			
	AGC	TGG	AAC	ATG	GTG	GAC	GTG	GAC	CTC	CCG	CCC	AAC	AAG	GAC	GTG	GAG	1734
	Ser	Trp	Asn	Met	Val	Asp	Val	Asp	Leu	Pro	Pro	Asn	Lys	Asp	Val	Glu	
			520					525						530			
50	CCC	GGC	ATC	TTA	CTA	CAT	GGG	CTG	AAG	CCC	TGG	ACT	CAG	TAC	GCC	GTT	1782
	Pro	Gly	Ile	Leu	Leu	His	Gly	Leu	Lys	Pro	Trp	Thr	Gln	Tyr	Ala	Val	
		535					540					545					

	TAC GTC AAG GCT GTG ACC CTC ACC ATG GTG GAG AAC GAC CAT ATC CGT Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile Arg 550 555 560 565	1830
5	GGG GCC AAG AGT GAG ATC TTG TAC ATT CGC ACC AAT GCT TCA GTT CCT Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val Pro 570 575 580	1878
	TCC ATT CCC TTG GAC GTT CTT TCA GCA TCG AAC TCC TCT TCT CAG TTA Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln Leu 585 590 595	1926
10	ATC GTG AAG TGG AAC CCT CCC TCT CTG CCC AAC GGC AAC CTG AGT TAC Ile Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn Leu Ser Tyr 600 605 610	1974
15	TAC ATT GTG CGC TGG CAG CGG CAG CCT CAG GAC GGC TAC CTT TAC CGG Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Tyr Arg 615 620 625	2022
	CAC AAT TAC TGC TCC AAA GAC AAA ATC CCC ATC AGG AAG TAT GCC GAC His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala Asp 630 635 640 645	2070
20	GGC ACC ATC GAC ATT GAG GAG GTC ACA GAG AAC CCC AAG ACT GAG GTG Gly Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys Thr Glu Val 650 655 660	2118
	TGT GGT GGG GAG AAA GGG CCT TGC TGC GCC TGC CCC AAA ACT GAA GCC Cys Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu Ala 665 670 675	2166
25	GAG AAG CAG GCC GAG AAG GAG GAG GCT GAA TAC CGC AAA GTC TTT GAG Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe Glu 680 685 690	2214
30	AAT TTC CTG CAC AAC TCC ATC TTC GTG CCC AGA CCT GAA AGG AAG CGG Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Lys Arg 695 700 705	2262
	AGA GAT GTC ATG CAA GTG GCC AAC ACC ACC ATG TCC AGC CGA AGC AGG Arg Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser Arg 710 715 720 725	2310
35	AAC ACC ACG GCC GCA GAC ACC TAC AAC ATC ACC GAC CCG GAA GAG CTG Asn Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu Leu 730 735 740	2358
	GAG ACA GAG TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC AAG GAG AGA Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Lys Glu Arg 745 750 755	2406
40	ACT GTC ATT TCT AAC CTT CGG CCT TTC ACA TTG TAC GCG ATC GAT ATC Thr Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Ala Ile Asp Ile 760 765 770	2454
45	CAC AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC AAC His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser Asn 775 780 785	2502
	TTC GTC TTT GCA AGG ACT ATG CCC GCA GAA GGA GCA GAT GAC ATT CCT Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile Pro 790 795 800 805	2550
50	GGG CCA GT ACC TGG GAG CCA AGG CCT GAA AAC TCC ATC TTT TTA AAG Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu Lys 810 815 820	2598

50

		TGG	CCG	GAA	CCT	GAG	AAT	CCC	AAT	GGA	TTG	ATT	CTA	ATG	TAT	GAA	ATA	2646
		Trp	Pro	Glu	Pro	Glu	Asn	Pro	Asn	Gly	Leu	Ile	Leu	Met	Tyr	Glu	Ile	
					825						830					835		
5		AAA	TAC	GGA	TCA	CAA	GTT	GAG	GAT	CAG	CGA	GAA	TGT	GTG	TCC	AGA	CAG	2694
		Lys	Tyr	Gly	Ser	Gln	Val	Glu	Asp	Gln	Arg	Glu	Cys	Val	Ser	Arg	Gln	
				840					845					850				
		GAA	TAC	AGG	AAG	TAT	GGA	GGG	GCC	AAG	CTA	AAC	CGG	CTA	AAC	CCG	GGG	2742
		Glu	Tyr	Arg	Lys	Tyr	Gly	Gly	Ala	Lys	Leu	Asn	Arg	Leu	Asn	Pro	Gly	
				855				860					865					
10		AAC	TAC	ACA	GCC	CGG	ATT	CAG	GCC	ACA	TCT	CTC	TCT	GGG	AAT	GGG	TCG	2790
		Asn	Tyr	Thr	Ala	Arg		Gln	Ala	Thr	Ser	Leu	Ser	Gly	Asn	Gly	Ser	
						875						880					885	
		TGG	ACA	GAT	CCT	GTG	TTC	TTC	TAT	GTC	CAG	GCC	AAA	ACA	GGA	TAT	GAA	2838
15		Trp	Thr	Asp	Pro		Val	Phe	Phe	Tyr	Val	Gln	Ala	Lys	Thr	Gly	Tyr	
					890							895				900		
		AAC	TTC	ATC	CAT	CTG	ATC	ATC	GCT	CTG	CCC	GTC	GCT	GTC	CTG	TTG	ATC	2886
		Asn	Phe	Ile	His	Leu	Ile	Ile	Ala	Leu	Pro	Val	Ala	Val	Leu	Leu	Ile	
					905					910					915			
20		GTG	GGA	GGG	TTG	GTG	ATT	ATG	CTG	TAC	GTC	TTC	CAT	AGA	AAG	AGA	AAT	2934
		Val	Gly	Gly	Leu	Val	Ile	Met	Leu	Tyr	Val	Phe	His	Arg	Lys	Arg	Asn	
				920					925					930				
		AAC	AGC	AGG	CTG	GGG	AAT	GGA	GTG	CTG	TAT	GCC	TCT	GTG	AAC	CCG	GAG	2982
		Asn	Ser	Arg	Leu	Gly	Asn	Gly	Val	Leu	Tyr	Ala	Ser	Val	Asn	Pro	Glu	
				935				940					945					
25		TAC	TTC	AGC	GCT	GCT	GAT	GTG	TAC	GTT	CCT	GAT	GAG	TGG	GAG	GTG	GCT	3030
		Tyr	Phe	Ser	Ala	Ala	Asp	Val	Tyr	Val	Pro	Asp	Glu	Trp	Glu	Val	Ala	
						955						960					965	
		CGG	GAG	AAG	ATC	ACC	ATG	AGC	CGG	GAA	CTT	GGG	CAG	GGG	TCG	TTT	GGG	3078
30		Arg	Glu	Lys	Ile	Thr	Met	Ser	Arg	Glu	Leu	Gly	Gln	Gly	Ser	Phe	Gly	
						970					975					980		
		ATG	GTC	TAT	GAA	GGA	GTT	GCC	AAG	GGT	GTG	GTG	AAA	GAT	GAA	CCT	GAA	3126
		Met	Val	Tyr	Glu	Gly	Val	Ala	Lys	Gly	Val	Val	Lys	Asp	Glu	Pro	Glu	
					985					990					995			
35		ACC	AGA	GTG	GCC	ATT	AAA	ACA	GTG	AAC	GAG	GCC	GCA	AGC	ATG	CGT	GAG	3174
		Thr	Arg	Val	Ala	Ile	Lys	Thr	Val	Asn	Glu	Ala	Ala	Ser	Met	Arg	Glu	
				1000					1005					1010				
		AGG	ATT	GAG	TTT	CTC	AAC	GAA	GCT	TCT	GTG	ATG	AAG	GAG	TTC	AAT	TGT	3222
		Arg	Ile	Glu	Phe	Leu	Asn	Glu	Ala	Ser	Val	Met	Lys	Glu	Phe	Asn	Cys	
				1015				1020					1025					
40		CAC	CAT	GTG	GTG	CGA	TTG	CTG	GGT	GTG	GTG	TCC	CAA	GGC	CAG	CCA	ACA	3270
		His	His	Val	Val	Arg	Leu	Leu	Gly	Val	Val	Ser	Gln	Gly	Gln	Pro	Thr	
						1035						1040					1045	
		CTG	GTC	ATC	ATG	GAA	CTG	ATG	ACA	CGG	GGC	GAT	CTC	AAA	AGT	TAT	CTC	3318
45		Leu	Val	Ile	Met	Glu	Leu	Met	Thr	Arg	Gly	Asp	Leu	Lys	Ser	Tyr	Leu	
						1050					1055					1060		
		CGG	TCT	CTG	AGG	CCA	GAA	ATG	GAG	AAT	AAT	CCA	GTC	CTA	GCA	CCT	CCA	3366
		Arg	Ser	Leu	Arg	Pro	Glu	Met	Glu	Asn	Asn	Pro	Val	Leu	Ala	Pro	Pro	
					1065					1070					1075			
50		AGC	CTG	AGC	AAG	ATG	ATT	CAG	ATG	GCC	GGA	GAG	ATT	GCA	GAC	GGC	ATG	3414
		Ser	Leu	Ser	Lys	Met	Ile	Gln	Met	Ala	ly	Glu	Ile	Ala	Asp	Gly	Met	
					1080				1085					1090				

	GCA TAC CTC AAC GCC AAT AAG TTC GTC CAC AGA GAC CTT GCT GCC CGG Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg 1095 1100 1105	3462
5	AAT TGC ATG GTA GCC GAA GAT TTC ACA GTC AAA ATC GGA GAT TTT GGT Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly 1110 1115 1120 1125	3510
	ATG ACG CGA GAT ATC TAT GAG ACA GAC TAT TAC CGG AAA GGA GGG AAA Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Arg Lys Gly Gly Lys 1130 1135 1140	3558
10	GGG CTG CTG CCC GTG CGC TGG ATG TCT CCT GAG TCC CTC AAG GAT GGA Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp Gly 1145 1150 1155	3606
15	GTC TTC ACC ACT TAC TCG GAC GTC TGG TCC TTC GGG GTC GTC CTC TGG Val Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp 1160 1165 1170	3654
	GAG ATC GCC ACA CTG GCC GAG CAG CCC TAC CAG GGC TTG TCC AAC GAG Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn Glu 1175 1180 1185	3702
20	CAA GTC CTT CGC TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCA GAC Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro Asp 1190 1195 1200 1205	3750
	AAC TGT CCT GAC ATG CTG TTT GAA CTG ATG CGC ATG TGC TGG CAG TAT Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln Tyr 1210 1215 1220	3798
25	AAC CCC AAG ATG AGG CCT TCC TTC CTG GAG ATC ATC AGC AGC ATC AAA Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser Ser Ile Lys 1225 1230 1235	3846
30	GAG GAG ATG GAG CCT GGC TTC CGG GAG GTC TCC TTC TAC TAC AGC GAG Glu Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr Tyr Ser Glu 1240 1245 1250	3894
	GAG AAC AAG CTG CCC GAG CCG GAG GAG CTG GAC CTG GAG CCA GAG AAC Glu Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu Pro Glu Asn 1255 1260 1265	3942
35	ATG GAG AGC GTC CCC CTG GAC CCC TCG GCC TCC TCG TCC TCC CTG CCA Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser Ser Leu Pro 1270 1275 1280 1285	3990
	CTG CCC GAC AGA CAC TCA GGA CAC AAG GCC GAG AAC GGC CCC GGC CCT Leu Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly Pro Gly Pro 1290 1295 1300	4038
40	GGG GTG CTG GTC CTC CGC GCC AGC TTC GAC GAG AGA CAG CCT TAC GCC Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln Pro Tyr Ala 1305 1310 1315	4086
45	CAC ATG AAC GGG GGC CGC AAG AAC GAG CGG GCC TTG CCG CTG CCC CAG His Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro Leu Pro Gln 1320 1325 1330	4134
	TCT TCG ACC TGC TGA TCCTTGGATC CTGAATCTGT GCAAACAGTA ACGTGTGCGC Ser Ser Thr Cys * 1335	4189
	ACGCGCAGCG GGGTGGGGGG GGAGAGAGAG TTTTAACAAT CCATTCACAA GCCTCCTGTA	4249
50	CCTCAGTGGG TCTTCAGTTC TGCCCTTGCT GCCCGCGGGA GACAGCTTCT CTGCAGTAA	4309

52

ACACATTTGG GATGTTCCCTT TTTTCAATAT GCAAGCAGCT TTTTATTCCC TGCCCAAACC 4369
 CTTAACTGAC ATGGGCCTTT AAGAACCTTA ATGACAACAC TTAATAGCAA CAGAGCACTT 4429
 GAGAACCAGT CTCCTCACTC TGTCCCTGTC CTCCCTGTT CTCCCTTTCT CTCTCCTCTC 4489
 TGCTTCATAA CGGAAAAATA ATTGCCACAA GTCCAGCTGG GAAGCCCTTT TTATCAGTTT 4549
 5 GAGGAAGTGG CTGTCCCTGT GGCCCCATCC AACCCTGTGA CACACCCGCC TGACACCGTG 4609
 GGTCAATTACA AAAAAACACG TGGAGATGGA AATTTTTACC TTTATCTTTC ACCTTTCTAG 4669
 GGACATGAAA TTTACAAAGG GCCATCGTTC ATCCAAGGCT GTTACCATT TAACGCTGCC 4729
 TAATTTTGCC AAAATCCTGA ACTTTCTCCC TCATCGGCCC GGCGCTGATT CCTCGTGTCC 4789
 GGAGGCATGG GTGAGCATGG CAGCTGGTTG CTCCATTTGA GAGACACGCT GGCGACACAC 4849
 10 TCCGTCCATC CGACTGCCCC TGCTGTGCTG CTCAAGGCCA CAGGCACACA GGTCTCATTG 4909
 CTTCTGACTA GATTATTATT TGGGGGAACT GGACACAATA GGTCTTTCTC TCAGTGAAGG 4969
 TGGGGAGAAG CTGAACCGGC 4989

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
 15 (A) LENGTH: 1368 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

20 Met Lys Ser Gly Ser Gly Gly Gly Ser Pro Thr Ser Val Trp Gly Leu
 -30 -25 -20 -15
 Leu Phe Leu Ser Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile
 -10 -5 1
 25 Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg
 5 10 15
 Leu Glu Asn Cys Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile
 20 25 30
 Ser Lys Ala Glu Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val
 35 40 45 50
 30 Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu
 55 60 65
 Gly Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe
 70 75 80
 35 Tyr Asn Tyr Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile
 85 90 95
 Gly Leu Tyr Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu
 100 105 110
 Lys Asn Ala Asp Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile
 115 120 125 130

53

	Leu	Asp	Ala	Val	Ser	Asn	Asn	Tyr	Ile	Val	Gly	Asn	Lys	Pro	Pro	Lys	
					135					140					145		
	Glu	Cys	Gly	Asp	Leu	Cys	Pro	Gly	Thr	Met	Glu	Glu	Lys	Pro	Met	Cys	
				150					155					160			
5	Glu	Lys	Thr	Thr	Ile	Asn	Asn	Glu	Tyr	Asn	Tyr	Arg	Cys	Trp	Thr	Thr	
			165					170					175				
	Asn	Arg	Cys	Gln	Lys	Met	Cys	Pro	Ser	Thr	Cys	Gly	Lys	Arg	Ala	Cys	
		180					185					190					
10	Thr	Glu	Asn	Asn	Glu	Cys	Cys	His	Pro	Glu	Cys	Leu	Gly	Ser	Cys	Ser	
		195				200					205					210	
	Ala	Pro	Asp	Asn	Asp	Thr	Ala	Cys	Val	Ala	Cys	Arg	His	Tyr	Tyr	Tyr	
				215						220					225		
	Ala	Gly	Val	Cys	Val	Pro	Ala	Cys	Pro	Pro	Asn	Thr	Tyr	Arg	Phe	Glu	
				230					235					240			
15	Gly	Trp	Arg	Cys	Val	Asp	Arg	Asp	Phe	Cys	Ala	Asn	Ile	Leu	Ser	Ala	
			245					250					255				
	Glu	Ser	Ser	Asp	Ser	Glu	Gly	Phe	Val	Ile	His	Asp	Gly	Glu	Cys	Met	
		260					265					270					
20	Gln	Glu	Cys	Pro	Ser	Gly	Phe	Ile	Arg	Asn	Gly	Ser	Gln	Ser	Met	Tyr	
		275				280					285					290	
	Cys	Ile	Pro	Cys	Glu	Gly	Pro	Cys	Pro	Lys	Val	Cys	Glu	Glu	Glu	Lys	
					295					300				305			
	Lys	Thr	Lys	Thr	Ile	Asp	Ser	Val	Thr	Ser	Ala	Gln	Met	Leu	Gln	Gly	
				310					315					320			
25	Cys	Thr	Ile	Phe	Lys	Gly	Asn	Leu	Leu	Ile	Asn	Ile	Arg	Arg	Gly	Asn	
			325					330					335				
	Asn	Ile	Ala	Ser	Glu	Leu	Glu	Asn	Phe	Met	Gly	Leu	Ile	Glu	Val	Val	
		340					345					350					
30	Thr	Gly	Tyr	Val	Lys	Ile	Arg	His	Ser	His	Ala	Leu	Val	Ser	Leu	Ser	
		355				360					365					370	
	Phe	Leu	Lys	Asn	Leu	Arg	Leu	Ile	Leu	Gly	Glu	Glu	Gln	Leu	Glu	Gly	
				375						380				385			
	Asn	Tyr	Ser	Phe	Tyr	Val	Leu	Asp	Asn	Gln	Asn	Leu	Gln	Gln	Leu	Trp	
				390					395					400			
35	Asp	Trp	Asp	His	Arg	Asn	Leu	Thr	Ile	Lys	Ala	Gly	Lys	Met	Tyr	Phe	
			405					410					415				
	Ala	Phe	Asn	Pro	Lys	Leu	Cys	Val	Ser	Glu	Ile	Tyr	Arg	Met	Glu	Glu	
		420					425					430					
40	Val	Thr	Gly	Thr	Lys	Gly	Arg	Gln	Ser	Lys	Gly	Asp	Ile	Asn	Thr	Arg	
		435				440					445					450	
	Asn	Asn	Gly	Glu	Arg	Ala	Ser	Cys	Glu	Ser	Asp	Val	Leu	His	Phe	Thr	
				455						460				465			
	Ser	Thr	Thr	Thr	Ser	Lys	Asn	Arg	Ile	Ile	Ile	Thr	Trp	His	Arg	Tyr	
				470					475					480			

54

	Arg	Pro	Pro	Asp	Tyr	Arg	Asp	Leu	Ile	Ser	Phe	Thr	Val	Tyr	Tyr	Lys
			485					490					495			
	Glu	Ala	Pro	Phe	Lys	Asn	Val	Thr	Glu	Tyr	Asp	Gly	Gln	Asp	Ala	Cys
		500					505					510				
5	Gly	Ser	Asn	Ser	Trp	Asn	Met	Val	Asp	Val	Asp	Leu	Pro	Pro	Asn	Lys
	515					520					525					530
	Asp	Val	Glu	Pro	Gly	Ile	Leu	Leu	His	Gly	Leu	Lys	Pro	Trp	Thr	Gln
					535					540					545	
10	Tyr	Ala	Val	Tyr	Val	Lys	Ala	Val	Thr	Leu	Thr	Met	Val	Glu	Asn	Asp
				550					555					560		
	His	Ile	Arg	Gly	Ala	Lys	Ser	Glu	Ile	Leu	Tyr	Ile	Arg	Thr	Asn	Ala
			565					570					575			
	Ser	Val	Pro	Ser	Ile	Pro	Leu	Asp	Val	Leu	Ser	Ala	Ser	Asn	Ser	Ser
		580					585					590				
15	Ser	Gln	Leu	Ile	Val	Lys	Trp	Asn	Pro	Pro	Ser	Leu	Pro	Asn	Gly	Asn
	595					600					605					610
	Leu	Ser	Tyr	Tyr	Ile	Val	Arg	Trp	Gln	Arg	Gln	Pro	Gln	Asp	Gly	Tyr
					615					620					625	
20	Leu	Tyr	Arg	His	Asn	Tyr	Cys	Ser	Lys	Asp	Lys	Ile	Pro	Ile	Arg	Lys
				630					635					640		
	Tyr	Ala	Asp	Gly	Thr	Ile	Asp	Ile	Glu	Glu	Val	Thr	Glu	Asn	Pro	Lys
			645					650					655			
	Thr	Glu	Val	Cys	Gly	Gly	Glu	Lys	Gly	Pro	Cys	Cys	Ala	Cys	Pro	Lys
		660					665					670				
25	Thr	Glu	Ala	Glu	Lys	Gln	Ala	Glu	Lys	Glu	Glu	Ala	Glu	Tyr	Arg	Lys
	675					680					685					690
	Val	Phe	Glu	Asn	Phe	Leu	His	Asn	Ser	Ile	Phe	Val	Pro	Arg	Pro	Glu
					695					700					705	
30	Arg	Lys	Arg	Arg	Asp	Val	Met	Gln	Val	Ala	Asn	Thr	Thr	Met	Ser	Ser
				710					715					720		
	Arg	Ser	Arg	Asn	Thr	Thr	Ala	Ala	Asp	Thr	Tyr	Asn	Ile	Thr	Asp	Pro
			725					730					735			
	Glu	Glu	Leu	Glu	Thr	Glu	Tyr	Pro	Phe	Phe	Glu	Ser	Arg	Val	Asp	Asn
		740				745						750				
35	Lys	Glu	Arg	Thr	Val	Ile	Ser	Asn	Leu	Arg	Pro	Phe	Thr	Leu	Tyr	Ala
	755					760					765					770
	Ile	Asp	Ile	His	Ser	Cys	Asn	His	Glu	Ala	Glu	Lys	Leu	Gly	Cys	Ser
					775					780					785	
40	Ala	Ser	Asn	Phe	Val	Phe	Ala	Arg	Thr	Met	Pro	Ala	Glu	Gly	Ala	Asp
				790					795					800		
	Asp	Ile	Pro	Gly	Pro	Val	Thr	Trp	lu	Pro	Arg	Pro	Glu	Asn	Ser	Ile
			805					810					815			
	Phe	Leu	Lys	Trp	Pro	Glu	Pro	Glu	Asn	Pro	Asn	Gly	Leu	Ile	Leu	Met
		820					825					830				

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Tyr Glu Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val
 835 840 845 850
 Ser Arg Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu
 855 860 865
 5 Asn Pro Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly
 870 875 880
 Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr
 885 890 895
 10 Gly Tyr Glu Asn Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val
 900 905 910
 Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg
 915 920 925 930
 Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val
 935 940 945
 15 Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp
 950 955 960
 Glu Val Ala Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly
 965 970 975
 20 Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp
 980 985 990
 Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser
 995 1000 1005 1010
 Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu
 1015 1020 1025
 25 Phe Asn Cys His His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly
 1030 1035 1040
 Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys
 1045 1050 1055
 30 Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met Glu Asn Asn Pro Val Leu
 1060 1065 1070
 Ala Pro Pro Ser Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala
 1075 1080 1085 1090
 Asp Gly Met Ala Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu
 1095 1100 1105
 35 Ala Ala Arg Asn Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly
 1110 1115 1120
 Asp Phe Gly Met Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys
 1125 1130 1135
 40 Gly Gly Lys Gly Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu
 1140 1145 1150
 Lys Asp Gly Val Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val
 1155 1160 1165 1170
 Val Leu Trp Glu Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu
 1175 1180 1185

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Ser Asn Glu Gln Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp
 1190 1195 1200
 Lys Pro Asp Asn Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys
 1205 1210 1215
 5 Trp Gln Tyr Asn Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser
 1220 1225 1230
 Ser Ile Lys Glu Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr
 1235 1240 1245 1250
 10 Tyr Ser Glu Glu Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu
 1255 1260 1265
 Pro Glu Asn Met Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser
 1270 1275 1280
 Ser Leu Pro Leu Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly
 1285 1290 1295
 15 Pro Gly Pro Gly Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln
 1300 1305 1310
 Pro Tyr Ala His Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro
 1315 1320 1325 1330
 20 Leu Pro Gln Ser Ser Thr Cys *
 1335

I CLAIM:

- 1 1. A method for increasing growth factor receptor gene
2 expression in a vertebrate, said method comprising the step
3 of exposing cells of said vertebrate to an ATG-directed
4 sense oligonucleotide, said oligonucleotide comprising at
5 least 14 nucleotides and being capable of stimulating
6 growth factor receptor gene expression.
- 1 2. The method of claim 1, wherein a composition comprising an
2 effective amount of an oligonucleotide having an ATG-
3 directed sense nucleotide sequence directed to a growth
4 factor receptor mRNA and being capable of stimulating
5 expression of said gene is applied to a target tissue, with
6 the result that angiogenesis is stimulated in said target
7 tissue.
- 1 3. The method of claim 2 wherein said target tissue is
2 affected by peripheral vascular disease.
- 1 4. The method of claim 2 wherein said target tissue is heart
2 tissue which has been affected by myocardial infarction.
- 1 5. A method of inhibiting vascular smooth muscle cell
2 proliferation in a coronary artery after a heart transplant
3 or after angioplasty in a vertebrate, said method
4 comprising the step of exposing the interior of said artery
5 to a composition comprising an antisense oligonucleotide of
6 at least 14 nucleotides, said oligonucleotide being
7 complementary to a portion of a mRNA for insulin-like
8 growth factor 1 receptor and capable of inhibiting
9 expression of insulin-like growth factor 1 receptor gene
10 expression.
- 1 6. A method of inhibiting angiogenesis in a target tissue,
2 said method comprising the step of administering to said
3 target tissue a composition which comprises a

4 therapeutically effective amount of an IGF IR-specific
5 antisense oligonucleotide or a recombinant nucleic acid
6 molecule which directs the synthesis of an IGF IR-specific
7 antisense RNA in said target tissue and a pharmaceutically
8 effective carrier.

1 7. The method of any of claims 1-6 wherein said growth factor
2 receptor gene is the insulin-like growth factor I receptor
3 (IGF 1R) gene.

1 8. The method of any of claims 6-7 wherein antisense
2 oligonucleotide comprises a nucleotide sequence as given in
3 SEQ ID NO:1 or SEQ ID NO:4.

1 9. The method of any of claims 1-8 wherein said
2 oligonucleotide is a phosphorothioate oligonucleotide, a
3 phosphorodithioate oligonucleotide, a phosphotriester
4 oligonucleotide, a methylphosphonate oligonucleotide, an
5 phosphoramidite oligonucleotide, an α -anomer
6 oligonucleotide, and a phosphoroselenoate oligonucleotide.

1 10. The method of any of claims 1-9 wherein said vertebrate is
2 rat or human.

1 11. The method of any of claims 1-9 wherein said vertebrate is
2 a species of swine, equine, bovine, ovine, caprine, feline
3 or canine.

1 12. The method of any of claims 1-5 and 9-12 wherein said ATG-
2 directed sense oligonucleotide comprises a nucleotide
3 sequence as given in SEQ ID NO:2.

1 13. An oligonucleotide comprising a nucleotide sequence as
2 given in SEQ ID NO:2.

1 14. A method of inhibiting vascular smooth muscle cell
2 proliferation in a coronary artery after a heart transplant

3 or after angioplasty in a vertebrate, said method
4 comprising the step of exposing the interior of said artery
5 to a composition comprising a non-naturally occurring DNA
6 molecule having portion capable of expressing an antisense
7 RNA complementary to at least about 100 nucleotides of a
8 Type I insulin-like growth factor receptor mRNA in vascular
9 smooth muscle cells and a pharmaceutically acceptable
10 carrier.

1 15. The method of claim 14 wherein said non-naturally occurring
2 recombinant DNA molecule is an adenovirus vector which has
3 been genetically engineered to express said antisense
4 oligonucleotide.

1 16. A non-naturally occurring recombinant DNA molecule
2 comprising a nucleotide sequence encoding mature insulin-
3 like growth factor 1 receptor from rat.

1 17. The non-naturally occurring recombinant DNA molecule of
2 claim 14 wherein said rat insulin-like growth factor 1
3 receptor protein (IGF IR) has an amino acid sequence as
4 given in SEQ ID NO: 9.

1 18. The non-naturally occurring recombinant DNA molecule of
2 claim 17 wherein said nucleotide sequence encoding mature
3 IGF IR (α subunit and β subunit) of rat is given in SEQ ID
4 NO: 8 from nucleotide 136 to nucleotide 4156.

1 19. A method of claim 1, said method comprising the step of
2 applying an effective amount of an oligonucleotide having
3 an ATG-directed sense nucleotide sequence directed to a
4 growth factor receptor mRNA and being capable of
5 stimulating expression of said gene and a pharmaceutically
6 acceptable carrier to vertebrate tissue, with the result
7 that one of wound healing, burn healing, bone healing and
8 collateral blood vessel development is stimulated.

FIGURE 1

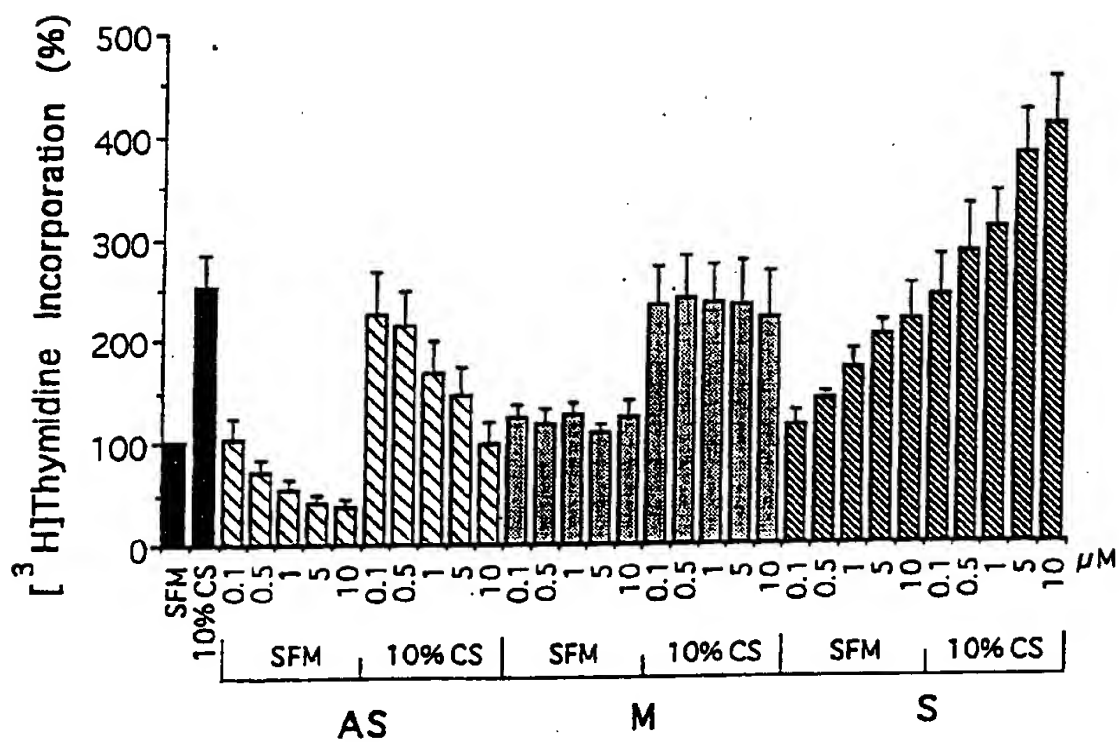


FIGURE 2

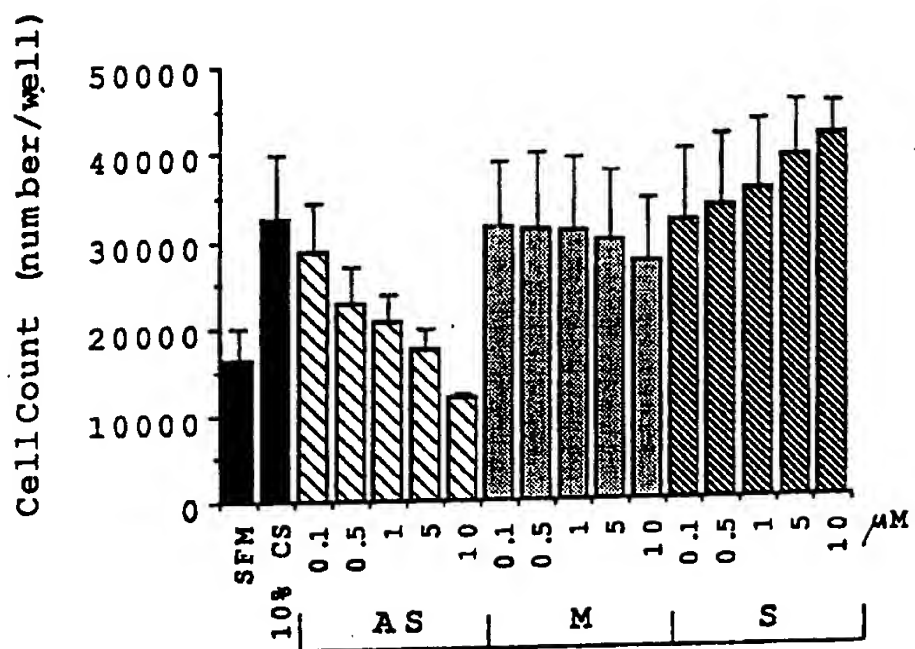


FIGURE 3

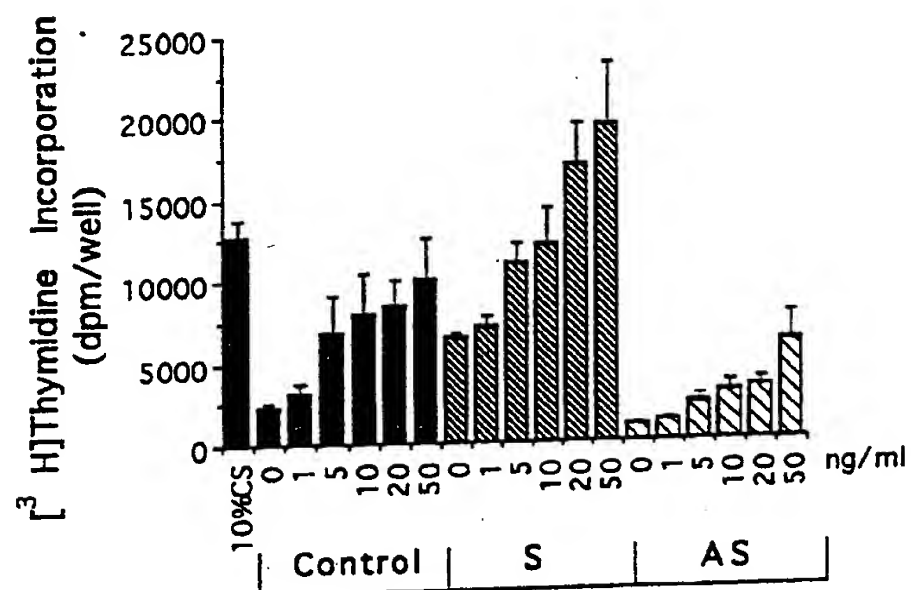


FIGURE 4

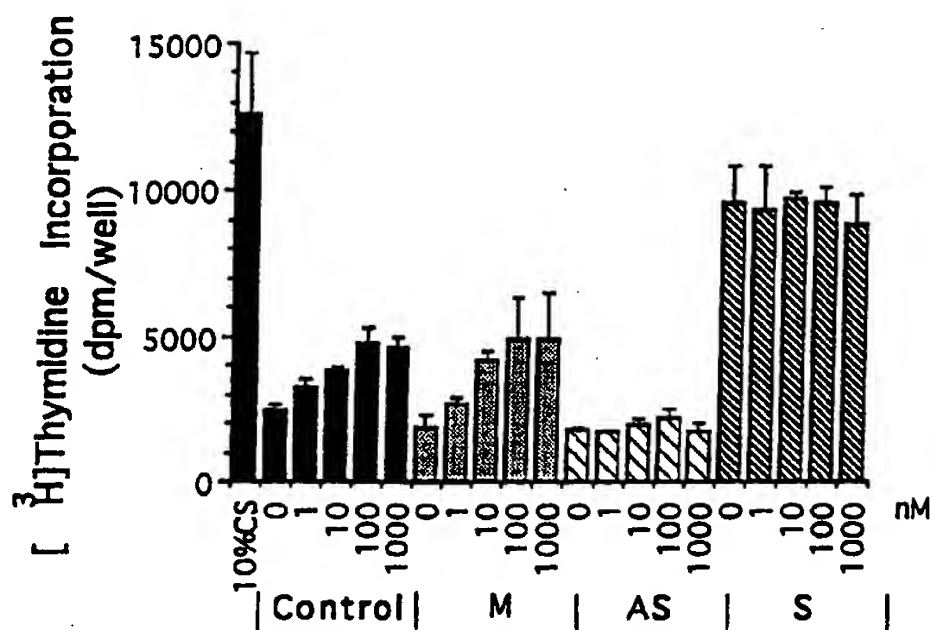


FIGURE 5

FIG. 5 A

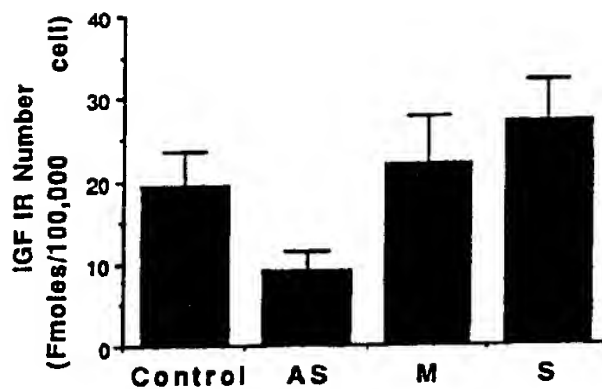


FIG. 5 B

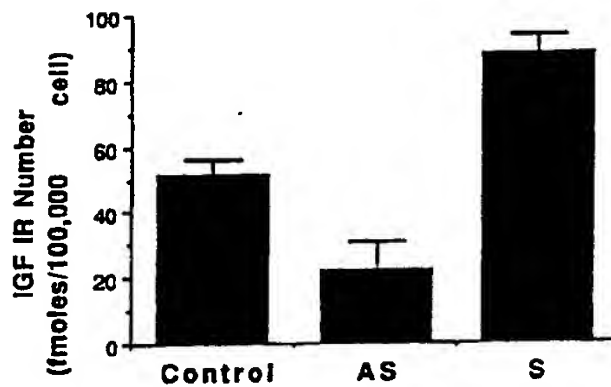


FIG. 5 C

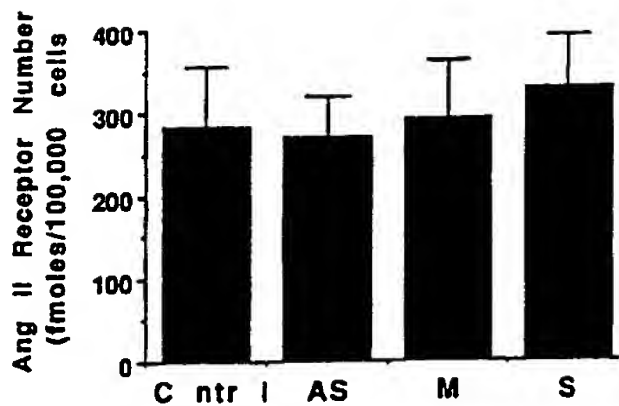


FIGURE 6

FIG. 6 A

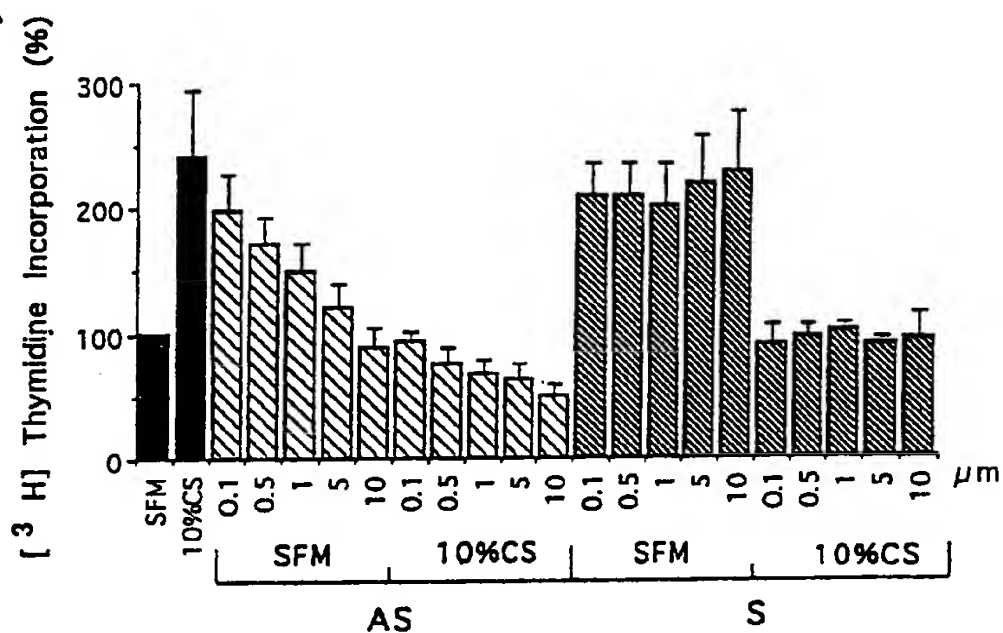


FIG. 6 B

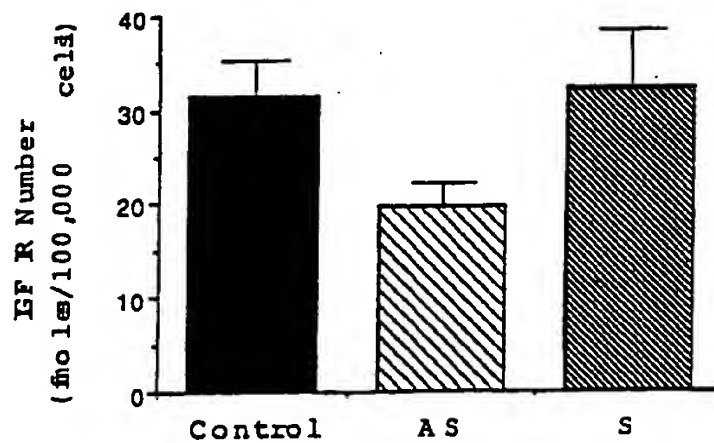


FIGURE 7

B

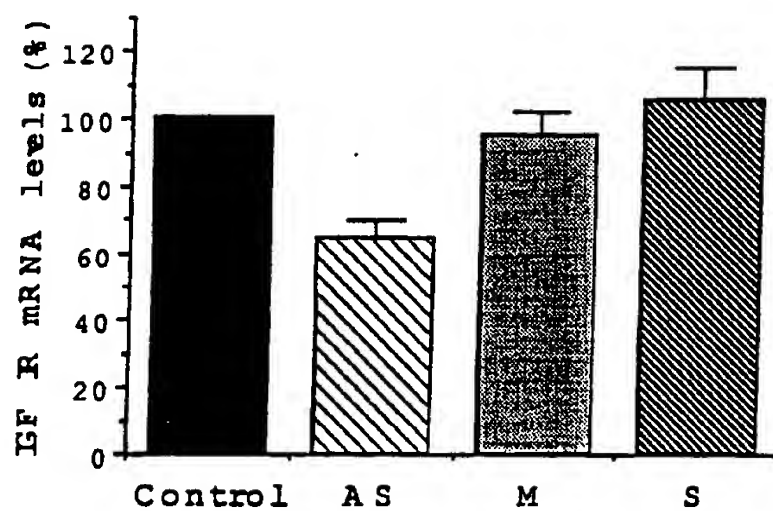


FIGURE 8

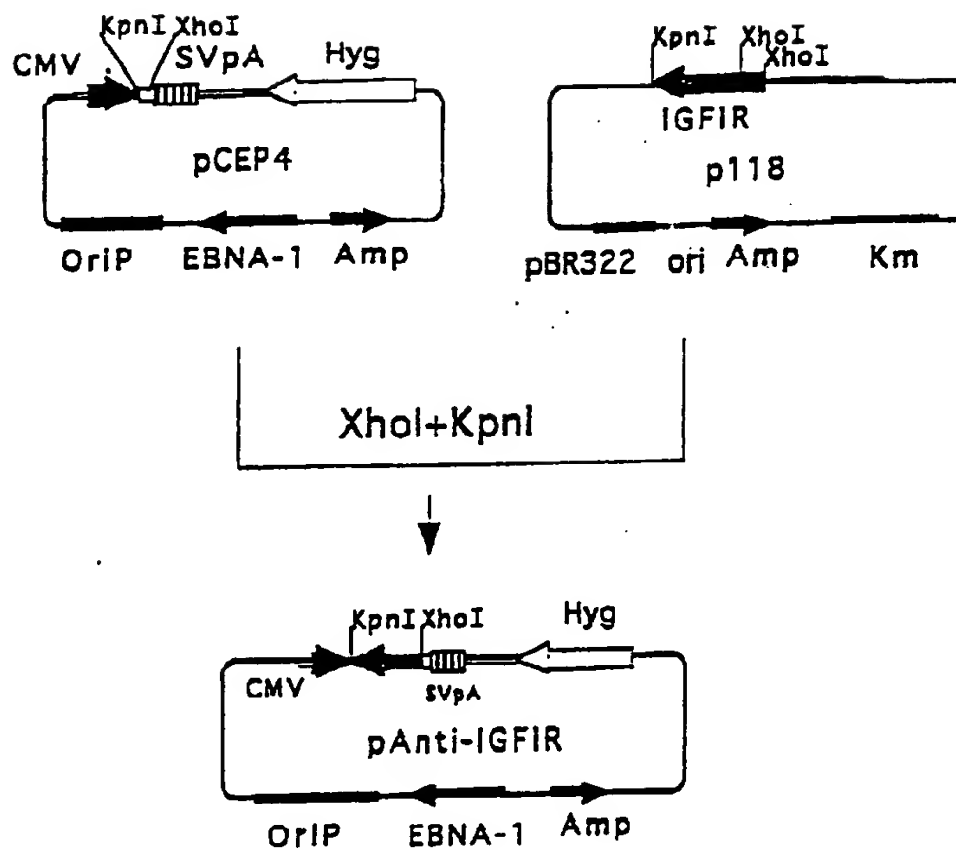


FIGURE 9

FIG. 9 A

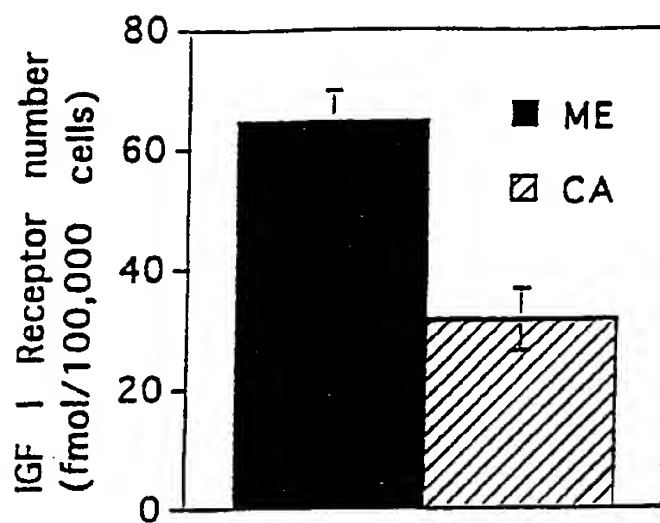


FIG. 9 B

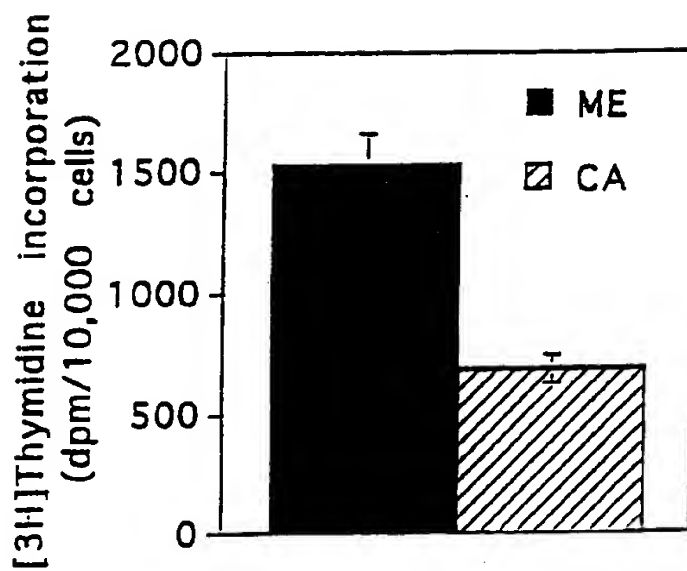
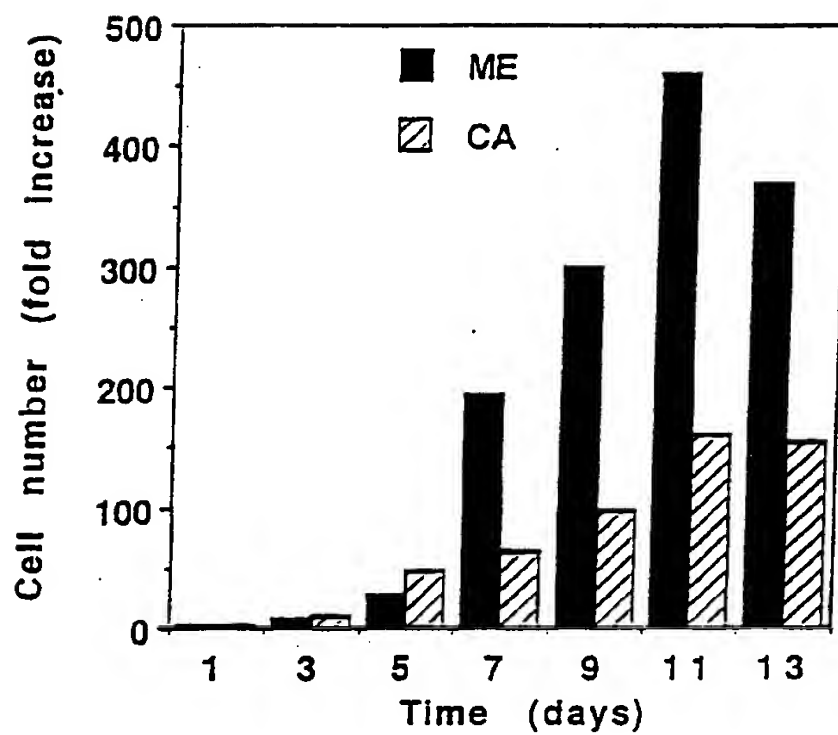


FIGURE 10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12563

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/00; C07H 21/04

US CL : 514/44; 536/23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,298,422 (SCHWARTZ ET AL) 29 MARCH 1994, see entire document.	1-15, 19
A,P	US, A, 5,434,134 (GLUCKMAN ET AL) 18 JULY 1995, see entire document.	1-4, 19
Y	WO, A, 93/09236 (BAYLOR COLLEGE OF MEDICINE) 13 MAY 1993, see entire document.	1-15, 19
A,P	WO, A, 94/22486 (THOMAS JEFFERSON UNIVERSITY) 13 OCTOBER 1994, see entire document.	5-18
Y	A. FLYVBJERG et al, "GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR I" published 1993 by WILEY & SONS, (Chichester, UK), pages 159-191, see entire document.	1-15, 19

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	A*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

04 DECEMBER 1995

Date of mailing of the international search report

01 FEB 1996

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Authorized officer

D. CURTIS HOGUE

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/12563

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TRENDS CARDIOVASC MED, Vol. 2, No. 3, issued 1992, Fagin et al, "Growth Factors, Cytokines, and Vascular Injury", pages 90-94, see entire document.	1-15, 19
Y	Proceedings of the American Association for Cancer Research, Vol. 33, issued March 1992, Shapiro et al, "Antisense-mediated reduction in insulinlike growth factor-I receptor expression suppresses the malignant phenotype of a human rhabdomyosarcoma", page 354, abstract 2112, see entire document.	5-15
Y	ARTERY, Vol. 18, No. 4, issued 1991, Ferns et al, "The Insulin-Like Growth Factors: Their Putative Role In Atherogenesis", pages 197-225, see entire document.	1-15, 19
Y	CLINICAL RESEARCH, Vol. 42, No. 2, issued 1994, Delafontaine et al, "Antisense Insulin-Like Growth Factor I Receptor Oligonucleotides Inhibit Vascular Smooth Muscle Cell Growth", page 305A, see entire document.	5-15
Y	JOURNAL OF CLINICAL INVESTIGATION, Vol. 94, issued 1994, Shapiro et al, "Antisense-mediated Reduction in Insulin-like Growth Factor-I Receptor Expression Suppresses the Malignant Phenotype of a Human Alveolar Rhabdomyosarcoma", pages 1235-1242, see entire document.	5-15
Y	AMERICAN JOURNAL OF CARDIOLOGY, Vol. 68, issued 04 November 1991, Cercek et al, "Growth Factors in Pathogenesis of Coronary Arterial Restenosis", pages 24C-33C, see entire document.	1-15, 19
Y	CURRENT OPINION IN CARDIOLOGY, Vol. 7, issued 1992, Neville et al, "The molecular biology of vein graft atherosclerosis and myointimal hyperplasia", pages 930-938, see entire document.	1-15, 19
Y	GROWTH REGULATION, Vol. 2, issued 1992, Florini et al, "Induction of Gene Expression in Muscle by the IGFs", pages 23-29, see entire document.	1-15, 19
Y,P	CIRCULATION RESEARCH, Vol. 76, issued 1995, Du et al, "Inhibition of Vascular Smooth Muscle Cell Growth Through Antisense Transcription of a Rat Insulin-Like Growth Factor I Receptor cDNA", pages 963-972, see entire document.	5-18
A	JOURNAL OF LEUKOCYTE BIOLOGY, Vol. 55, issued March 1994, Sunderkotter et al, "Macrophages and angiogenesis", pages 410-422, see entire document.	1-4, 19